Additional file 1

Supplementary Methods

Live/dead staining to evaluate slice health

Slice health was assessed by calcein/ethidium homodimer staining. Briefly, the confetti and slice were placed on a glass slide and incubated in calcein (4 μM) and ethidium homodimer (6 μM) to label live/dead cells respectively, for 15 minutes at 37°C in 5% CO_2/95% humidified air. Staining for slice health was performed at 4, 7, 14 and 21 days and, in the case of one sample subset, at 40 days. Slice cytoarchitecture and LIVE/DEAD proportions were assessed from fluorescence micrographs visualised using an Axio Scope A1 fluorescence microscope, AxioCam ICc1 digital camera and Axiovision software (Carl Zeiss MicroImaging, GmbH, Germany). To estimate slice viability, we used a previously reported technique (Weightman et al., 2014) to compare the integrated density of calcein staining (as a measure of overall viable cells) versus the integrated density of ethidium homodimer staining (as a measure of overall dead cells) in counterpart fluorescent images from the same field. The viable proportion was then calculated as a percentage of the total integrated density (live + dead). Four different fields were taken for each slice analysed. Analysis was conducted using ImageJ 1.53k (National Institutes of Health, Bethesda, MD, USA). All data are presented as mean ± standard error of the mean (SEM) where each slice was considered a separate “n” number.

Immunocytochemistry

To assess tissue architecture and the relationship with the DuraGen Plus™ insert, slices were prepared either for immunocytochemistry or transmission electron microscopy (TEM). Slices were immunostained to detect major neural cell types: astrocytes (GFAP); neurons (TuJ-1); microglia (Iba-1) and oligodendrocytes (MBP). Briefly, confetti and fixed slice were placed on a glass slide and subject to a Tris-buffered saline (TBS) wash prior to incubation for 1 hr at RT in NDS blocker (5% NDS; 0.3% Triton-x 100; 0.2% gelatin). Slices were then incubated in the specified primary antibody for 48 hours at 4°C. Following TBS washes (5 min/wash; 3×), slices were then incubated, protected from light, in NDS blocker containing the corresponding FITC/Cy3-labelled secondary antibody for 2 hours at room temperature. To remove residual stain, slices were TBS washed (5 min/wash; 3×) and allowed to air dry for 4 hours at room temperature before being mounted with DAPI, covered and the edges of the coverslip sealed to prevent desiccation of the tissue. Micrographs of the lesion-DuraGen™ Plus interface were captured using Leica MZ8 microscope (Leica Microsystems, Wetzlar, Germany). Deltapix InfinityX digital camera with InfinityX Analyze software (Release 6.5); Lumenera Corporation, Ottawa, Canada.

Transmission electron microscopy

For electron microscopy, a subset of slices at 21 days post-lesion were glutaraldehyde fixed for 2 hours (2.5% glutaraldehyde diluted in 0.1 M sodium cacodylate buffer/2 mM calcium chloride) and washed in sodium cacodylate buffer prior to post-fixation by incubation for 1 hour with 1% OsO_4. In brief, following fixation the tissue was taken through a step-wise ethanol (ETOH) dehydration process (70% ETOH – 24 hours; 90% ETOH – 24 hours × 2; 100% ETOH – 24 hours × 2; 100% DRY ETOH – 4 hours; 100% DRY ETOH – 48 hours). Slices were then embedded in Spurr[32] resin via 1:1 resin:dry ETOH, 3:1. resin:ETOH and several changes of pure resin, before polymerisation at 60°C for 16$^+$ hours. The area of the lesion and insert were trimmed down and ultrathin sections (100 nm) of sample were cut using a Reichert Ultracut E Microtome and collected on 200-mesh thin bar copper grids. Sections were stained using 2% (w/v, saturated) uranyl acetate in 70% ETOH and 2% (w/v) aqueous lead citrate before being examined in a JEOL-100CX 1230 TEM (Welwyn Garden City, Hertfordshire, UK), operating at an accelerating voltage of 100 kV. Digital images of cellular ultrastructure and biomaterial were acquired at varying magnifications using a megaview III camera system (EMSIS GmbH, Münster, Germany) and Olympus analySIS® software (Southend-on-Sea, UK).