Dexamethasone reduces the foreign body response to intraneural electrode implants in the peripheral nerve of the rat

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Running title: Dexamethasone for FBR to intraneural devices

Abstract

Intraneural electrodes must be in intimate contact with nerve fibers to have a proper function, but this interface is compromised due to the foreign body reaction (FBR). The FBR is characterized by a first inflammatory phase followed by a second anti-inflammatory and fibrotic phase, which results in the formation of a tissue capsule around the device, causing physical separation between the active sites of the electrode and the nerve fibers. We have tested several anti-inflammatory drugs such as dexamethasone, ibuprofen and maraviroc to reduce macrophage activation, as well as clodronate liposomes to reduce monocyte/macrophage infiltration, and sildenafil as an antifibrotic drug to reduce collagen deposition in an FBR model with longitudinal Parylene C intraneural devices implanted in the rat sciatic nerve. Treatment with dexamethasone, ibuprofen or clodronate significantly reduced the inflammatory response in the nerve in comparison to the saline group after two weeks of the implant, whereas sildenafil and maraviroc had no effect on infiltration of macrophages in the nerve. However, only dexamethasone was able to significantly reduce the matrix deposition around the implant. Similar positive results were obtained with dexamethasone in the case of polyimide-based intraneural implants, another polymer substrate for the electrode. These results indicate that inflammation triggers the FBR in peripheral nerves, and that anti-inflammatory treatment with dexamethasone may have beneficial effects on lengthening intraneural interface functionality.

Keywords: dexamethasone, foreign body response, intraneural electrodes, polyimide, parylene C.

Introduction

Advanced neuroprostheses are intended to link the human nervous system with electronic or robotic prostheses, with the aim of restoring motor and sensory functions lost after neural injuries or amputations. Various neuroprosthetic systems include an interface with the peripheral nerve. Although different peripheral nerve interfaces have been developed, all of them are based on their capability to contact with specific groups of axons within a nerve to obtain neural signals and to stimulate their activity, thus achieving a bidirectional communication (Navarro et al. 2005; del Valle and Navarro 2013). Interfaces designed to achieve a high resolution, in terms of recording the small amplitude of neural signals and of stimulation with high selectivity small fascicles of axons with a common target, require a close positioning of axons and electrode. Hence, intraneural electrodes have been proposed as showing an adequate trade-off thanks to a reduced invasiveness and a good selectivity (Micera et al. 2008). The most used designs of electrodes that are implanted within a peripheral nerve include the multielectrode arrays (MEAs), the longitudinal intra-fascicular electrode (LIFE) and the transversal intraneural multichannel electrode (TIME), all of them providing several (ten to hundred) small electrical active sites in one device. Compared with extraneural electrodes, such as cuff types, intraneural electrodes are placed within a fascicle in the nerve and have closer contact to the targeted axons. This intraneural positioning increases the signal-to-noise ratio of recordings, reduces the stimulus intensity needed to depolarize the axons, and enhances selectivity (Badia et al. 2011).

However, any implanted device in the body induces a host response, known as the foreign body reaction (FBR), that may ultimately compromise the long-term functional outcome of a biomedical device. The FBR is the response of the immune system to any external device implanted in the body (Ward 2008; Onuki et al. 2008). It is characterized by an initial inflammatory phase, followed by an inflammatory resolution and tissue remodeling phase that results in the formation of a fibrous tissue capsule around the device (Luttikhuizen et al. 2006; Anderson et al. 2008). In the case of an intraneural electrode, this encapsulation results in a progressive decline in signal level and increase in stimulation threshold, because the scar tissue gradually separates axons away from the contacts (Lago et al. 2007; Rossini et al. 2010; Christensen et al. 2014; Raspopovic et al. 2014; Wurth et al. 2017).

To improve the long-term functional outcome of implanted electrodes, different strategies have been developed. From the biomaterials field, different polymers (Sommakia et al. 2014; Skousen et al. 2014; Lee et al. 2017) and surface coatings (Cui et al. 2001; Balaji et al. 2015; Noorisafa et al. 2016) have been investigated to reduce the FBR. In fact, these coatings have been used as local delivery systems of active molecules or drugs to modulate the inflammatory response in subcutaneous (Norton et al. 2007; Hetrick et al. 2007) and brain

(Mercanzini et al. 2010; Zhong et al. 2017) implants. Besides, a combination of conductive polymers and anti-inflammatory treatment have shown better recording properties and closer neurons to implants over time (Boehler et al. 2017). However, only a few of these strategies have been applied to peripheral nerve interfaces, such as cuff (Vince et al. 2005; Heo et al. 2016b) and regenerative (FitzGerald 2016) devices. In the case of intraneural interfaces, there are some limitations (i.e. nerve size) that limit the use of bulky local administration strategies. Moreover, the effective time window to improve the FBR for intraneural electrodes is still unknown.

We have recently characterized the extent of the inflammatory and the remodeling phases of the FBR to devices longitudinally implanted in the peripheral nerve (De la Oliva et al., 2017), thus determining possible targets for reducing the encapsulation and improving electrode functional outcome. The aim of this study is to evaluate the effect of different treatments to modulate the FBR and to reduce capsule thickness around longitudinal parylene C intraneural devices inserted in the rat sciatic nerve. Hence, anti-inflammatory drugs such as dexamethasone, ibuprofen and maraviroc were administered for 2 weeks to reduce macrophage activation, as well as clodronate liposomes to reduce monocyte/macrophage infiltration. Sildenafil was also assayed as an antifibrotic drug to reduce collagen deposition around the device (Percival et al. 2012). The effects have been assessed in terms of anatomical and histological measures of the capsule and the infiltrating cells. Finally, the effects of dexamethasone have been also evaluated in the FBR to polyimide electrode implants, for comparing the differences between device substrates.

Material and Methods

Surgical procedures and drug administration

All animal experiments conducted were performed with the approval of the Ethical Committee of the Universitat Autònoma de Barcelona in accordance with the European Communities Council Directive 2010/63/EU.

Female Sprague-Dawley rats of 200-250 g of weight were used. Surgery was performed under ketamine and xylazine anaesthesia (90/10 mg/kg, i.p.). The implantation procedure was performed as previously described for LIFEs (Lago et al. 2007). Briefly, the sciatic nerve was surgically exposed at the midthigh and freed from surrounding adherences. Parylene C and polyimide strips of 20 mm length, 200 µm width and 10 µm thickness, mimicking the structure of the thin-film LIFE (Lago et al. 2007) were longitudinally implanted in the tibial branch of the sciatic nerve with the help of a straight needle attached to a 10-0 loop thread (STC-6, Ethicon). A longitudinal implant was chosen because of its better reproducibility in comparison with transversal implants and to better study only the FBR inside the nerve. Finally, the surrounding muscles and the skin were sutured and disinfected with povidone-iodine. Animals were housed at 22±2ºC on a 12h light-dark cycle with food and water ad libitum.

Treatments to reduce FBR to the parylene C intraneural devices started 2 days before surgery to ensure appropriate systemic levels and were daily administered for 2 weeks since we have previously found that at this time postimplant the thickness of the encapsulation reaches stable maximal values (De la Oliva et al., 2017). We have assayed three anti-inflammatory drugs, dexamethasone, ibuprofen and maraviroc, to reduce macrophage activation; clodronate liposomes to reduce monocyte/macrophage infiltration, and sildenafil to reduce collagen deposition around the device. Animal distribution, doses and administration pathways are summarized in Table 1. Dosage was selected according to previous reports in the literature. In a second part of the study, the most effective drug treatment found, i.e. dexamethasone, was administered at three different doses to assess dose-effect response. Finally, dexamethasone was administered to rats with either parylene C or polyimide implants for comparing possible differences between electrode substrates, and during 1, 2 or 8 weeks for comparing the differences in duration of treatment.

For each drug and material group, a saline group was studied as control. However, as there were no differences between administration pathways, all the saline animals were combined in a unique group for parylene or polyimide devices.

Morphological evaluation

After 2 or 8 weeks post-implant, animals were deeply anaesthetized with an overdose of pentobarbital and transcardially perfused with 4% PFA in phosphate buffer (PB) to obtain the sciatic nerve including the implanted device for histological and immunohistochemical analyses.

To evaluate the microstructure of the nerve and the thickness of the tissue capsule around the device, nerve segments were postfixed in 3% glutaraldhyde-3% paraformaldehyde and postfixed in 2% OsO4 for 2h, dehydrated through ethanol series and embedded in epon resin. Semithin sections (0.5 µm thick) were stained with toluidine blue and examined by light microscopy. The thickness of the tissue capsule was measured as the distance between each side of the device and the closest myelinated axon using ImageJ software.

Analysis of infiltrating macrophages in the implanted nerves was performed using immunohistochemical labeling. Nerve segments containing the device implanted were serially cut (15 µm) with a cryostat (Leica CM190, Leica Microsystems). After thawing and blocking with normal donkey serum, slides were incubated overnight at 4ºC with primary antibody rabbit anti-Iba1 (Wako, 191947, 1:500). Slides were then washed with 0.1% Tween 20 buffer solution and incubated with AlexaFluor 555 donkey anti-rabbit secondary antibody (Invitrogen, A21207, 1:200) for 1 h at room temperature. Finally, slides were mounted with Mowiol containing DAPI (0.1 µg/ml, Sigma). The number of Iba1 positive macrophages that infiltrate the whole tibial nerve was quantified in images taken with an epifluorescence microscope (Olympus BX51). Images were equally treated to adjust brightness and contrast, and background subtraction. A threshold of detection and binarization was applied using ImageJ software. Iba1 positive cells in the whole tibial nerve, excluding the implant and the tissue capsule, were counted using the plugin “Analyze particles” of ImageJ.

Hematoxylin-eosin staining was performed to evaluate the formation of foreign body giant cells (FBGCs) as a result of macrophage fusion around the implant. Other cryostat sections were immersed in hematoxylin Harrys solution (Fluka, Sigma) for 7 min, washed in water and immersed in 1% HCl in ethanol for 20 sec. Then, sections were washed again in water and stained with Eosin Y (Merck Millipore) for 5 min. Finally, sections were dehydrated with series of graded ethanol rinses and mounted with DPX (Sigma). The number of FBGCs in each stained section was counted under the microscope and results expressed as FBGC per mm of implant width. In addition, the diameter of each FBGC counted was measured using ImageJ.

Statistical analysis

All reported values are the average ± SEM. Differences between groups or times postimplant were analyzed by one or two-way ANOVA followed by Bonferroni post hoc tests, using the GraphPad Prism software. Statistical significance was considered at $p < 0.05$.

Results

Anti-inflammatory treatment reduces the infiltration of macrophages in implanted nerves

The infiltration of macrophages in parylene C implanted nerves was significantly reduced by treatment with ibuprofen, clodronate and dexamethasone compared to the saline administered group (Fig. 1). In contrast, maraviroc and sildenafil did not reduce the number of macrophages present after 2 weeks of treatment. The combination of dexamethasone and sildenafil significantly reduced the infiltration of iba1+ cells to similar levels than dexamethasone alone (Fig. 1).

As a hallmark of the final phase of the FBR, the number of FBGCs was evaluated. These cells are the result of the fusion of macrophages when they are not able to phagocyte the foreign body and are associated with an anti-inflammatory and tissue remodeling environment (McNally et al. 2011; Sheikh et al. 2015). No changes in the number of FBGCs around the implant were found for any of the treatments administered except for maraviroc (Fig. 2), which caused a marked increase in FBGCs in the capsule around the devices in comparison to saline-treated animals. No changes in the diameter of these cells were observed between groups (Fig. 2).

The FBR results in the formation of a tissue capsule around the implanted devices in the peripheral nerve. Among the anti-inflammatory drugs used only dexamethasone significantly reduced the thickness of the capsule compared with the saline controls (Fig. 3). Sildenafil also reduced the formation of this capsule, although to a lesser degree. The combination of both treatments, dexamethasone plus sildenafil during 2 weeks did not show any additive effect.

Since dexamethasone was the most effective drug in reducing inflammatory cell infiltration and encapsulation of the intraneural devices, we further investigated the dose-effect relation. Thus, dexamethasone was given daily at 400, 200 and 50 µg/kg in different groups of rats. The results showed that the lowest dose was not able to reduce significantly cell infiltration and tissue encapsulation, whereas the highest and the medium doses were similarly effective (Fig.4).

Evaluation of dexamethasone effect on the FBR to different polymers

We also tested the effects of systemic dexamethasone administration on the FBR to intraneural devices made of polyimide, another polymer used as electrode substrate (Lago et al. 2007; Rodriguez et al. 2000; Lacour et al. 2009). As expected, dexamethasone treatment also reduced the infiltration of iba1+ macrophages in polyimide implanted nerves and the tissue capsule thickness around the device (Fig. 5). Comparatively, the characteristics of the FBR were similar for both parylene C and polyimide substrates. The beneficial effect of

dexamethasone was slightly more marked for the polyimide device, particularly in the limitation of macrophage infiltration after 2 weeks.

Finally, to know whether the reduction in macrophage infiltration and tissue deposition persisted over time after treatment withdrawal to avoid secondary adverse effects, groups of rats were administered for either 1, 2 or 8 weeks with dexamethasone at 200 µg/kg/s.i.d. and followed for 2 or 8 weeks of implant. The analyses made at 2 weeks after implant showed that only 1 week of treatment was not enough to reduce macrophage infiltration nor thickness of the capsule around both polyimide and parylene C devices (Fig. 5). On the contrary, the results after 8 weeks of implant showed that only 2 weeks of dexamethasone administration were sufficient to reduce the capsule thickness, even though, treatment maintained for 8 weeks resulted in a more marked effect for both materials (Fig. 6).

Discussion

The results of this study demonstrate that dexamethasone is effective in reducing the inflammatory reaction and the fibrous encapsulation seen as the response to electrodes implanted in the peripheral nerve. This is an important issue in the application of nerve interfaces for neuroprostheses because progressive scarring will impair long-term interface function. Even with recent advances in the electrode design to make them more adaptive and stable (Lago et al., 2007; Boretius et al., 2010; Cutrone et al., 2015) and in the use of biocompatible materials (Sommakia et al. 2014), the FBR still compromises the long-term functional performance of implanted neural interfaces.

In this study, systemic administration of different drugs has been tested to reduce the tissue encapsulation of longitudinal intraneural devices. Dexamethasone has been found as the most effective treatment to modulate the FBR, by reducing the infiltration of macrophages and the capsule thickness around both parylene C and polyimide devices. Our results agree with those previously found with systemic (Spataro et al. 2005) and local (Zhong and Bellamkonda 2007; FitzGerald 2016) administration of dexamethasone, in which a reduction of scar tissue formation was reported. Moreover, improvement in electrode functionality due to local release of dexamethasone has also been described (Kim and Martin 2006; Mercanzini et al. 2010). However, most of the local release studies have been made on brain (Kim and Martin 2006; Zhong and Bellamkonda 2007; Mercanzini et al. 2010) and subcutaneous implants (Dang et al. 2011; Kastellorizios et al. 2015; Heo et al. 2016a). Only a few works have focused on strategies to focally release anti-inflammatory drugs in peripheral nerves (Park et al. 2015; FitzGerald 2016; Heo et al. 2016b). Indeed, by using systemic administration we aimed to make a feasible screening of different drugs with the potential to prevent the FBR. Eventually, modifications in the design of current intraneural electrodes may allow to include systems for focal, controlled release, either by coating their surface with biodegradable hydrogels or by adapting a microfluidic system. Nevertheless, the volume limits within the peripheral nerve must be considered, so these options should avoid an increase in the thickness of the electrode.

On the other hand, systemic administration facilitates the combination of different FBR modulatory drugs at different time points after implantation, according to the time-window of effectiveness for each drug. As our results show, at least two weeks of dexamethasone administration (at a dose of 200 µg/kg/s.i.d.) are needed to significantly reduce the infiltration of immune cells and the thickness of the capsule. The effect achieved during the first two weeks is maintained after 8 weeks of implant, suggesting that this initial period is predictive of the later chronic FBR. Despite the reduction of the FBR to electrode polymers (i.e. parylene C and polyimide) obtained with dexamethasone, still some encapsulation appeared around the

implanted devices. Therefore, the ultimate question is how this reduction in capsule thickness may improve the functionality of intraneural interfaces in terms of stimulation and nerve signals recording. Current experiments will determine the potential effect of dexamethasone in improving the functional characteristics of chronically implanted intraneural electrodes. Given the similarities in the FBR with other types of devices, such as multielectrode arrays (Christensen et al. 2014) and transversal electrodes (Wurth et al. 2017), dexamethasone treatment will be likely useful to modulate the FBR to different electrode designs for peripheral nerves.

The differences found between the several anti-inflammatory drugs assayed in this work point out the mechanisms through which dexamethasone is doing its positive effect. Ibuprofen is a NSAID, known to exert anti-inflammatory effect by the inhibition of cyclooxygenase 1 and 2 enzymes. Clodronate induces apoptosis of circulating monocytes/macrophages. Dexamethasone acts through the glucocorticoid receptor, having a much wider spectrum of actions (Coutinho and Chapman 2011), from anti-inflammatory to immunosuppressive effects. Thus, the reduction of infiltrating macrophages and the decrease of immune mediators achieved also with ibuprofen and clodronate treatments, would not be enough to reduce the encapsulation of implanted devices. In the same line, maraviroc, an antagonist of CCR5, did not reduce macrophages infiltration nor tissue deposition in the implanted nerves. Despite the role of CCR5 in different fibrotic models (Seki et al. 2009; Berres et al. 2010; Sahin and Wasmuth 2013), we did not observe any beneficial effect of its blockage in our model of FBR in peripheral nerves. However, CCR5 is not the only chemokine receptor implicated in tissue fibrosis (Ishida et al. 2007; Sahin and Wasmuth 2013).

As expected from previous studies using sildenafil for reducing fibrosis development (Valente et al. 2003; Noel et al. 2012; Percival et al. 2012), we found a significant reduction in capsule thickness. However, the effect was not as marked as with dexamethasone. This could be due to the two main phases of the FBR, one pro-inflammatory followed by a second one anti-inflammatory and tissue remodeling (Anderson et al. 2008). Thus, dexamethasone might be modulating both phases while the other treatments assessed only act by reducing either activation of the inflammatory cells or the matrix deposition.

In conclusion, systemic administration of dexamethasone could be a feasible option to improve the long-term usability of neural electrodes in peripheral nerves without modifications on electrodes design. Further advances can be expected if the FBR might be modulated according to the cellular and molecular players within each phase, with sequential administration of appropriate drugs for each phase.

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Tables

Table 1. Drugs, doses and administration pathways used in this work.

Group	Material	Treatment duration	Dose	Administration	N
Dexamethasone	ParC	2w	400 µg/kg/s.i.d.[1]	s.c	6
			200 µg/kg/s.i.d.[1]		12
			50 µg/kg/s.i.d.[1]		6
		1w	200 µg/kg/s.i.d.[1]		8
		2w			8
		8w			8
	Pol	1w	200 µg/kg/s.i.d.[1]		8
		2w			8
		8w			8
Ibuprofen	ParC	2w	30 mg/kg/s.i.d.[1]	p.o.	6
Clodronate	ParC	2w	10 ml/kg/q.a.d.[2]	i.v.	6
Sildenafil	ParC	2w	25 mg/kg/s.i.d.[1]	p.o.	6
Dex+Sild	ParC	2w		s.c., p.o.	6
Maraviroc	ParC	2w	30 mg/kg/b.d.s.[3]	p.o.	10
Saline	ParC			s.c/p.o/i.v.	28
Saline	Pol			s.c.	8

[1]s.i.d.: once a day, [2]q.a.d.: every other day, [3]b.d.s.: twice a day.

Figure legends

Figure 1. Representative images of iba1 labeled infiltrating macrophages in the tibial nerve 2 weeks after implant of a parylene electrode device, following administration of (A) saline, (B) ibuprofen, (C) clodronate, (D) maraviroc, (E) dexamethasone, (F) sildenafil or (G) dexamethasone and sildenafil. (H) Quantification of the number of iba1 positive cells in the nerve after the different treatments. Scale bar = 150µm. * p< 0.05 vs saline group.

Figure 2. Foreign body giant cells (FBGCs) in the capsule around the parylene electrode device. Changes in (A) number and (B) diameter of FBGCs after 2 weeks under the different treatments. * p< 0.05 vs saline group.

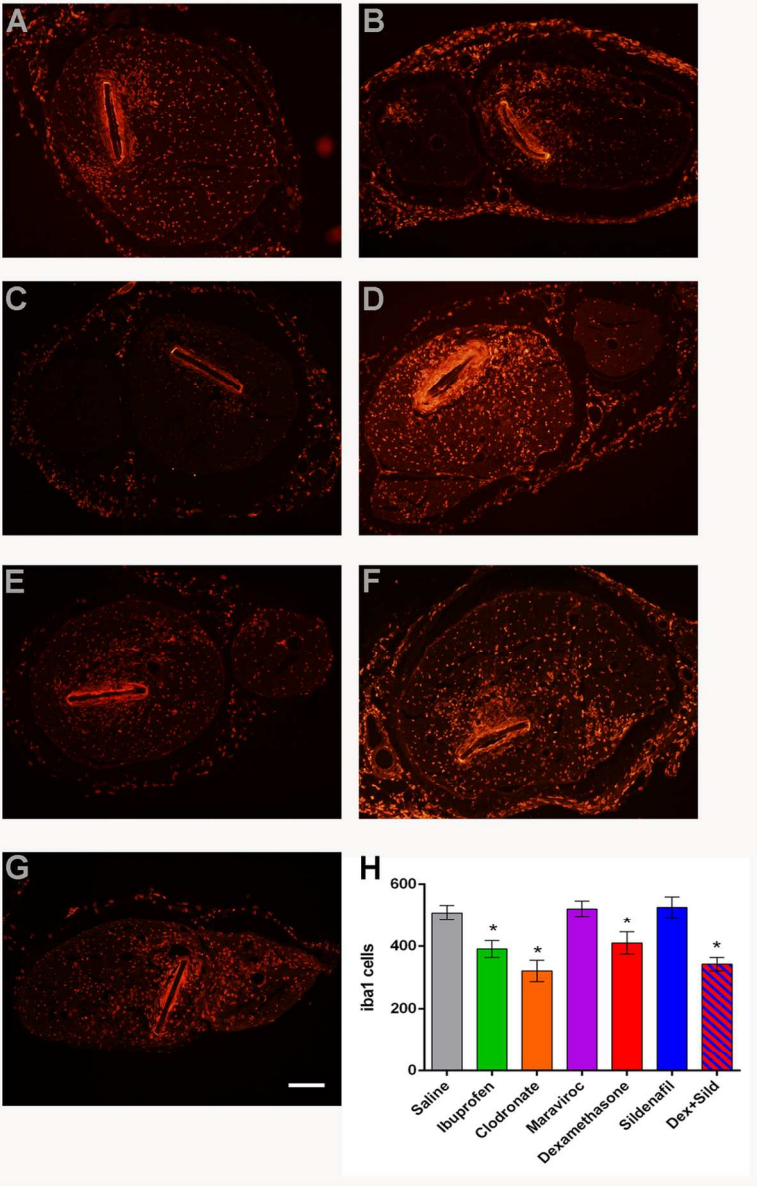
Figure 3. Tissue capsule formed around the parylene device with (A) saline, (B) ibuprofen, (C) clodronate, (D) maraviroc, (E) dexamethasone, (F) sildenafil or (G) combined dexamethasone and sildenafil treatment for 2 weeks. (H) Histogram of the capsule thickness. Scale bar = 50µm. * p< 0.05, ** p< 0.01 vs saline group.

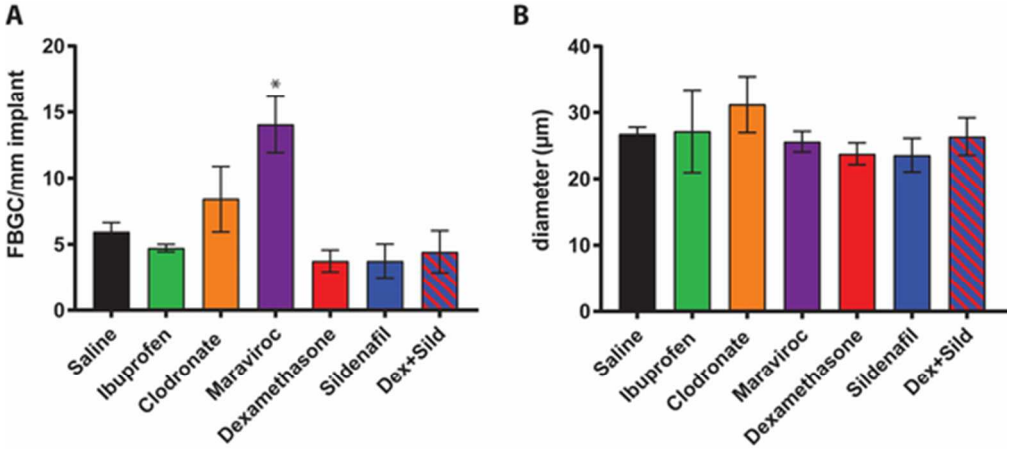
Figure 4. Dose-response effect of dexamethasone on (A) iba1+ cells infiltration, and (B) capsule formation around parylene C devices implanted in the nerve during 2 weeks. * p< 0.01 vs saline group.

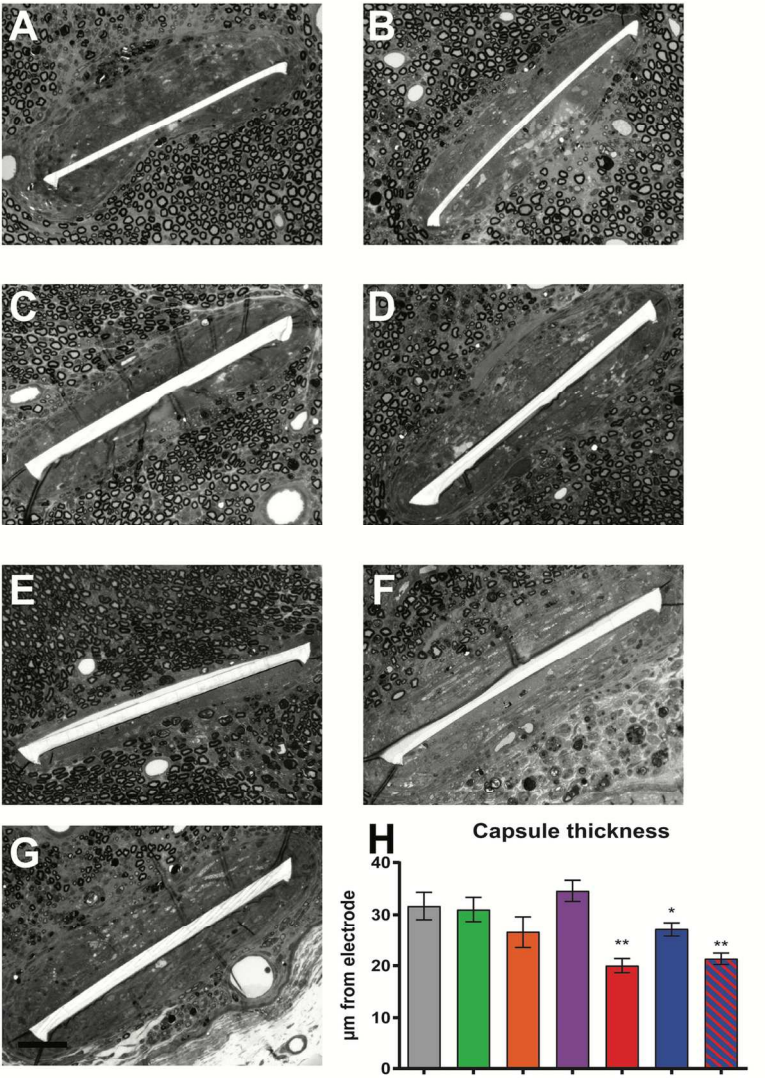
Figure 5. Effects of dexamethasone on FBR to parylene C and polyimide devices after 2 weeks implantation. (A) Representative images of iba1 labeling after 1 or 2 weeks of dexamethasone treatment in parylene C and polyimide implanted nerves. Scale bar = 100µm. (B) Quantification of iba1 positive cells after treatment with saline (Sal) or dexamethasone (Dex) during 2 (left) or 1 (right) week following intraneural implantation of parylene (blue) or polyimide (orange) devices. *p< 0.05 vs respective saline group. #p<0.05 vs respective 1+1w treated group. (C) Representative images of tissue capsule after 2 or 1 week of dexamethasone treatment around parylene C and polyimide devices implanted in the nerve. Scale bar = 100µm. (D) Capsule thickness measurement after treatment with saline (Sal) or dexamethasone (Dex) during 2 (left) or 1 (right) week in parylene (blue) or polyimide (orange) devices. * p< 0.05 vs respective saline group. # p<0.05 vs respective 1+1w treated group.

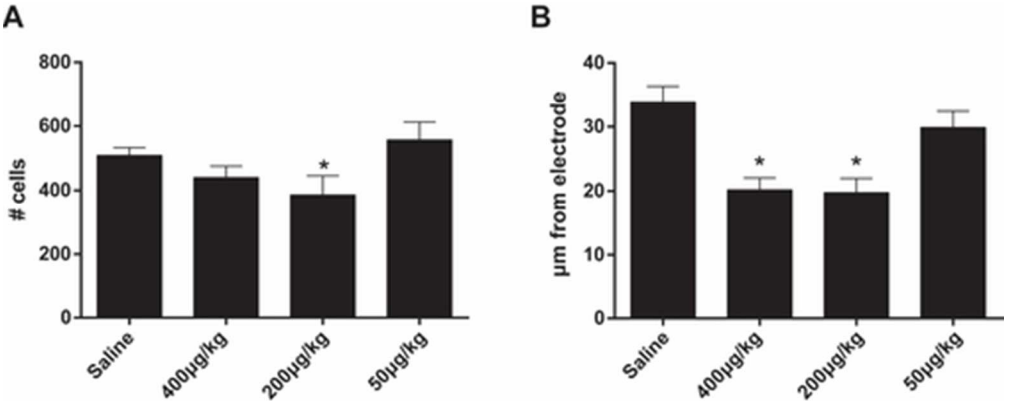
Figure 6. Effects of dexamethasone on FBR to parylene C and polyimide devices after 8 weeks implantation. (A) Representative images of iba1 labeling after 2 or 8 weeks of dexamethasone treatment in parylene C and polyimide implanted nerves. Scale bar = 100µm. (B) Quantification

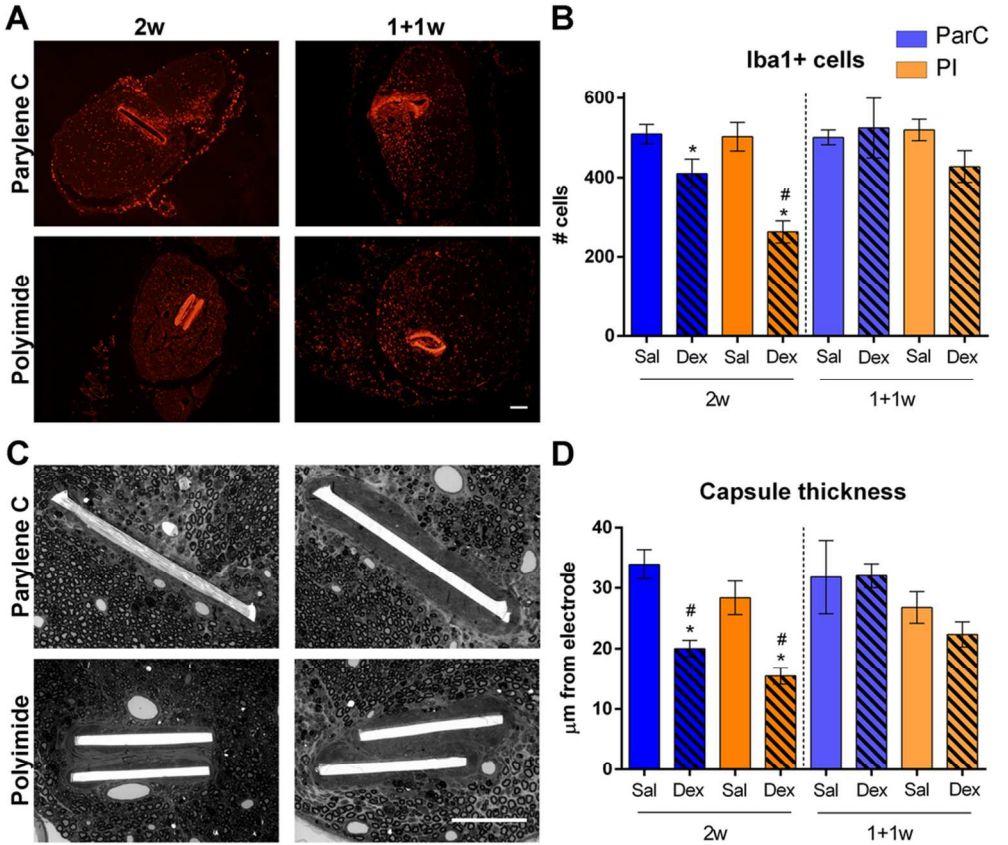
of iba1 positive cells after treatment with saline (Sal) or dexamethasone (Dex) during 8 (left) or 2 (right) weeks after parylene (blue) or polyimide (orange) devices implant. *p< 0.05 vs respective saline group. (C) Representative images of tissue capsule after 2 or 8 weeks of dexamethasone treatment in parylene C and polyimide implanted devices. Scale bar = 100µm. (D) Capsule thickness measurement after treatment with saline (Sal) or dexamethasone (Dex) during 8 (left) or 2 (right) weeks around parylene (blue) or polyimide (orange) implanted devices. * p< 0.05 vs respective saline group. # p<0.05 vs respective 2+6w treated group.

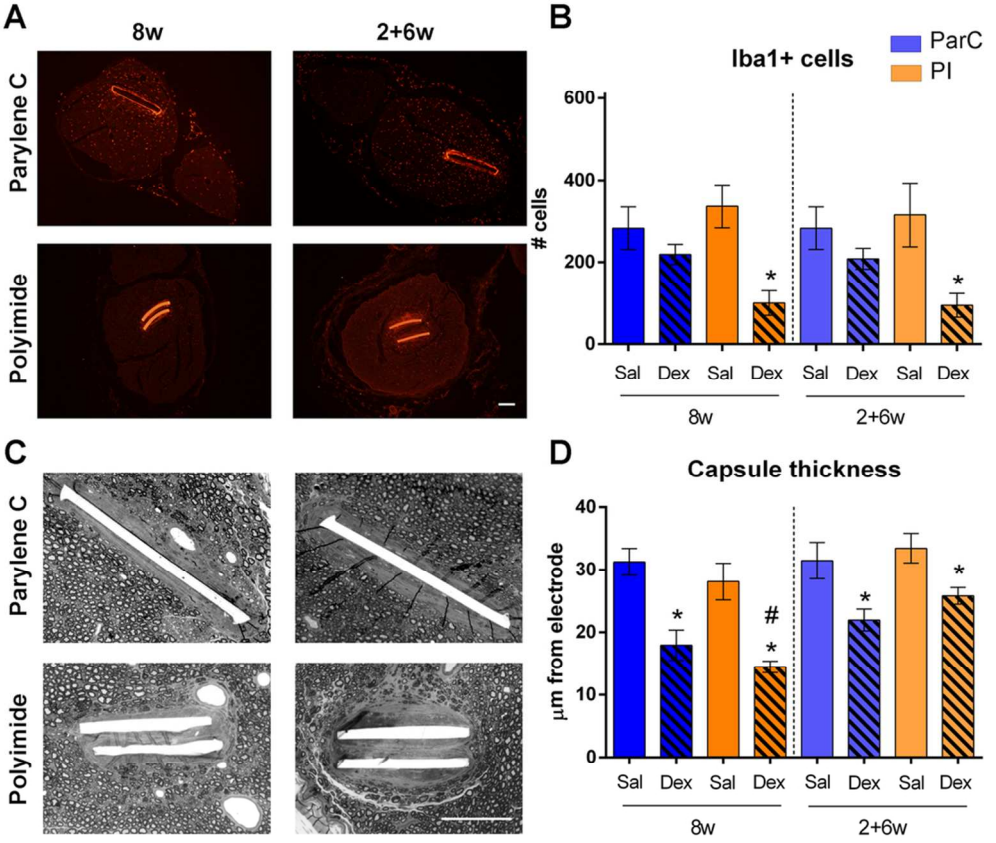

A
B
C
D
E
F
G
H
iba1 cells
600
400
200
0
*
*
*
*
Saline
Ibuprofen
Clodronate
Maraviroc
Dexamethasone
Sildenafil
Dex+Sild


A
FBGC/mm implant
20
15
10
5
0
*
Saline
Ibuprofen
Clodronate
Maraviroc
Dexamethasone
Sildenafil
Dex+Sild
B
diameter (µm)
40
30
20
10
0
Saline
Ibuprofen
Clodronate
Maraviroc
Dexamethasone
Sildenafil
Dex+Sild


A
B
C
D
E
F
G
H
Capsule thickness
µm from electrode
40
30
20
10
0
**
*
**
Saline
Ibuprofen
Clodronate
Maraviroc
Dexamethasone
Sildenafil
Dex+Sild


A
800
600
400
200
0
cells
*
Saline
400µg/kg
200µg/kg
50µg/kg
B
40
30
20
10
0
µm from electrode
*
*
Saline
400µg/kg
200µg/kg
50µg/kg


A
2w
1+1w
Parylene C
Polyimide
B
Iba1+ cells
ParC
PI
cells
600
400
200
0
Sal
Dex
Sal
Dex
Sal
Dex
Sal
Dex
2w
1+1w
C
Parylene C
Polyimide
D
Capsule thickness
µm from electrode
40
30
20
10
0
Sal
Dex
Sal
Dex
Sal
Dex
Sal
Dex
2w
1+1w


A
8w
2+6w
Parylene C
Polyimide
B
Iba1+ cells
ParC
PI
cells
600
400
200
0
Sal
Dex
Sal
Dex
Sal
Dex
Sal
Dex
8w
2+6w
C
Parylene C
Polyimide
D
Capsule thickness
μm from electrode
40
30
20
10
0
Sal
Dex
Sal
Dex
Sal
Dex
Sal
Dex
8w
2+6w