Quantification of LPS Eluate from Coated Microelectrode Devices

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Introduction:

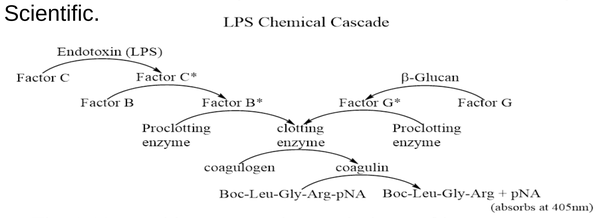
Penetrating microelectrode arrays have a great potential to be used as control and communication interfaces for neuroprosthetics. However, a persistent obstacle in the clinical implementation of microelectrode arrays is the chronic degradation of these devices, putatively due to the foreign body response. Though researchers have studied the progression of the foreign body response and the effect of anti-inflammatory drugs on the efficacy of the implant, the exact biological mechanisms of implant degradation are not fully understood.

Accelerated implant failure is a valuable approach to more closely investigating the effect of the foreign body response on device degradation (Otto,Williams). We propose to exacerbate the immune response by coating the devices with lipopolysaccharide (LPS) – a cell wall component of bacteria which induces inflammation. Quantifying the amount of LPS released from a coated electrode is crucial in performing such an experiment. Using a Limulus amebocyte lysate (LAL) test – a test based on the extract of the blood from horseshoe crab which reacts with LPS – the concentration of LPS can be quantified, allowing for a more careful characterization of the inflammatory response.

Methods:

LPS Quantification:

The LAL test used was performed under the instructions provided by Thermo Scientific.





- The LAL test kit was used on solutions of known concentration to create the standard curve between .1EU/mL and 1EU/mL where 1 endotoxin unit (EU) ≈ 1ng.
- The presence of LPS on devices which underwent different sterilization methods was compared using the LAL test kit.
- Devices were coated with 1mg/mL concentration and the coat was allowed to release into endotoxin free water. The concentration of the eluate was then observed using the LAL kit.

***In Vivo* Experiment:**

1. Following aseptic procedure, Single shank Michigan type dummy electrode was implanted in barrel cortex of mice.
2. For LPS treated animals, device was dipped in 1 mg/ml LPS solution prior to implantation. For Dex group, 200ug/kg of Dexamethasone – an anti-inflammatory drug – was injected subcutaneously a day before and after the surgery.
3. Blood was collected retro-orbitally at three time points (1 day before, 2 days after and 1 week after the surgery).
4. Collected serum was analyzed using Meso Scale Discovery's (MSD) Proinflammatory Panel to test the levels of cytokines.

Basis:

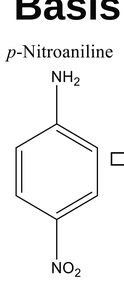


Figure 1.a

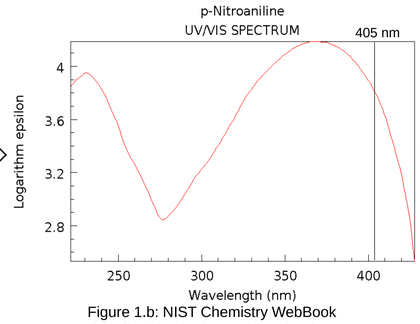


Figure 1.b: NIST Chemistry WebBook (http://webbook.nist.gov/chemistry)

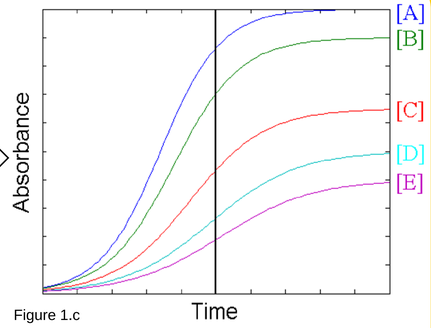


Figure 1.c

Figure 1.a-c: The LAL test relies on the production of *pNA* (1.a) which absorbs at 405nm (1.b). Different concentrations of LPS produce pNA at different rates, which can be seen when observing the absorbance at 405nm (figure 1.c).

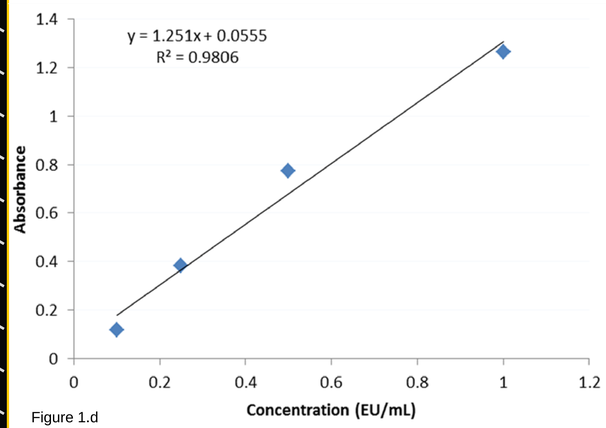


Figure 1.d

Figure 1.d: The Standard Curve is developed by performing the LAL assay on 50µL of known concentrations (.1, .25, .5, and 1EU/mL). Each data point is the average of triplicate readings. From the data points, a linear least squares regression resulted in a $r^2 = .9806$. In other words, 98.06% of the variation in absorbance is accounted for by the variation in concentration. Using the standard curve, one can determine the LPS concentration of an unknown sample through interpolation. The curve is accurate for concentrations within the range .1 – 1EU/mL.

In Vitro Results:

1 mg/mL eluate (EU)			
	range	average	uncertainty
1st elution	0.767	7.521	0.384
2nd elution	0.352	2.671	0.176
3rd elution	0.512	3.465	0.256
Total	5.388	4.552	2.694

Table 1: Three devices were coated in 1mg LPS/mL solution and then immediately immersed in 100µL endotoxin-free water to elute the LPS coating. The elutions were assessed using the LAL assay (n=3). Each elution was found to be measured fairly precise (relative uncertainty for each elution was < 10%). However, the range among all elutions (n=9) was 5.388EU, revealing the scatter among the three device elutions. The large spread can likely be attributed to the coating paradigm.

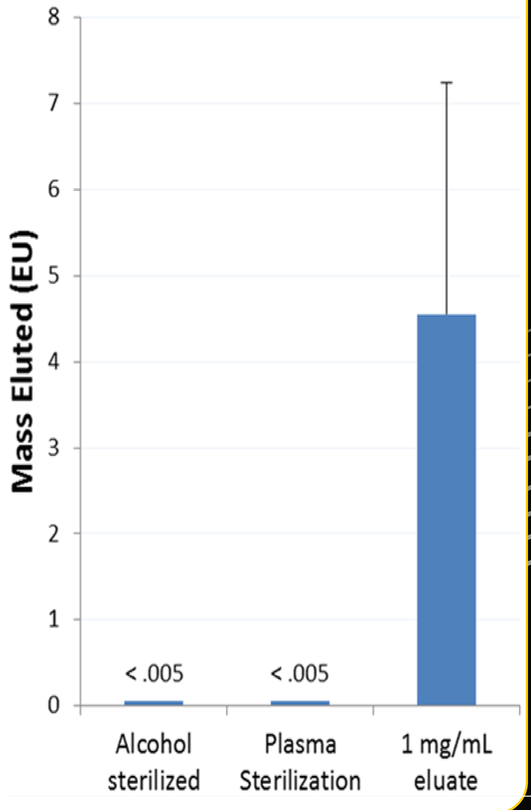


Figure 2: In order to be more certain of how much the LPS coating contributed to the eluate concentration, devices were also tested for LPS after sterilization techniques. Devices that were tested after sterilization were immersed in 50µL endotoxin-free water and the eluate was then tested for the presence of LPS. Both devices undergoing alcohol sterilization and plasma sterilization resulted in eluates with concentrations < .1 EU/mL

In Vivo Results:

Figure 3.a

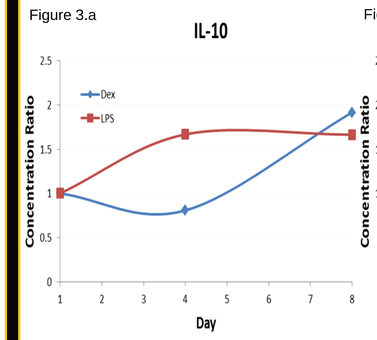


Figure 3.b

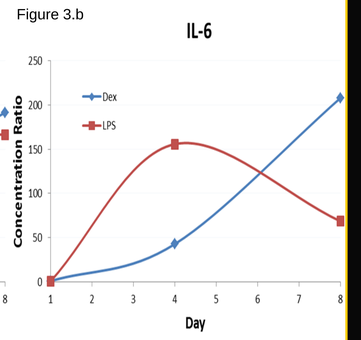


Figure 3.c

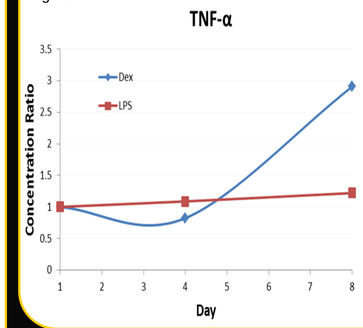


Figure 3: Fig 3a-c show the concentration of three pro-inflammatory and anti-inflammatory cytokines relative to Day 1 at Days 1, 4, and 8 (n=3). The upregulation of IL-6 is prominent in both the LPS and Dex treated test subjects (3.b). It seems the LPS mice may have had a more rapid response than the Dex treated. IL-6 inhibits the production of pro-inflammatory TNF-α, and activates production of anti-inflammatory IL-10. As the Dex treated mice experienced an upregulation of IL-6, they also experienced a noticeable increase in TNF- α as the inflammation seems to be worsening (3.c). The opposite is seen in the LPS treated mice. During the week, IL-10 seems to have upregulated slightly in both Dex and LPS treated mice (3.a).

Conclusions:

- Devices coated in 1mg/ml concentration of LPS eluted a mean mass of 4.55 ± 2.964EU, where 1 endotoxin unit (EU) ≈ 1ng.
- The large spread of the data can likely be attributed to variation in the coating paradigm (hand-guided coatings).
- These results indicate that LPS elution can be accurately measured using the LAL assay.
- The *In Vivo* results suggest that the introduction of LPS and Dex has a noticeable effect on the inflammatory response of mice.
- The use of Dex seems to delay the acute inflammatory response while LPS accelerates the process.
- Histology is currently in progress.

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