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Stabilizing electrode-host interfaces: A tissue engineering approach

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Abstract—The stability of implanted electrodes is a significant problem affecting their long-term use in vivo. Problems include mechanical failure and inflammation at the implantation site. The engineering of bioactive electrode coatings has been investigated for its potential to promote in-growth of neural tissue and reduce sheer at the electrode-host interface. Preliminary results indicate that hydrogel coatings with either collagen I or polylysine-laminin-1 can promote cortical nerve cell attachment and differentiation on silicon substrates. Additionally, slow-release microtubules can also be implanted in these gels to release agents that either provide trophic support to neurons or prevent inflammation locally. When silicon discs are coated with collagen type I, the coating remains stable for 55 days. Further testing is underway, but initial results indicate that tissue-engineering approaches provide useful insights to help address the problem of host-electrode instability in the brain.

Key words: *biomaterials, drug delivery, electrode coatings, FES, hydrogels, neural tissue engineering.*

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INTRODUCTION

The stability of implanted electrodes is a significant problem limiting current electrical recording and stimulation strategies in vivo. Potential reasons for this instability include the fact that most electrodes are made from silicon, which is a less-than-ideal material to implant into the human body. The biomaterials community has known this for years, but as yet, no other conducting polymers exist that can be reliably used, so silicon remains the material of choice. Because silicon causes electrodes to be nonadhesive, it is difficult for cells to grow on this material, even in vivo. Also, because of the mechanical mismatch, implanted electrodes are likely to produce sheer-induced inflammation in the chronic state.

In the field of tissue engineering, tissues are seen as organized groups of cells. If it were possible to mimic the molecular conditions and mechanisms biology employs to create this organization of cells, it might also be possible to engineer functional tissue, be it in vitro to grow artificial skin or in vivo at the implant site of a biomaterial. This paper will explore the possibility of applying tissue-engineering strategies to address the problem of long-term electrode instability in vivo. It is postulated that electrode coatings can be engineered to promote

in-growth of neural tissue and reduce inflammation and long-term instability at the electrode-host interface.

DESIGN CRITERIA FOR NEURAL-INTEGRATIVE COATINGS

Electrode coatings that reduce mechanical shear at the implantation site must be stable for long periods of time. They should contain agents capable of reducing inflammation and promoting neuronal survival, when nerve damage results from stimulation or from electrochemical reactions. Thus, the coatings should have at least two components. In the areas where electrical activation is not needed, there should be a porous neural adhesive component that promotes cell attachment, in-growth, and differentiation. Also embedded in this coating should ideally be slow-release mechanisms that actively manage the host-electrode interface by diffusion and slow-release trophic factors and/or anti-inflammatory agents. In addition, such a neurointegrative coating should be relatively thin, only 100 mm or so, so as not to interfere with the electrode's capability to record from or stimulate target cells. The rationale for the choice of materials to be used in these coatings is derived from work done in the fields of nerve regeneration and fetal development.

RELEVANCE OF REGENERATION RESEARCH

For many years bioengineers have considered ways to bridge the gap in severed nerves. The focus has been on the use of biomimetic materials instead of cellular transplants. Synthetic biomaterials can elude many of the problems inherent with the use of cellular materials, such as the source of the cells, host-donor compatibility, and cellular transport and storage. However, if the properties and characteristics of the cells that make them desirable candidates for transplantation were known and if it were possible to recreate that cellular apparatus with the use of materials in combination with scaffolds and drug delivery, then biomimetic materials could offer a preferable alternative to tissue transplants.

In addition to physically connecting the proximal and distal ends of a nerve, bridge materials must facilitate the survival of the nerve cell body and be conducive for the in-growth of regenerating nerve fibers. In addition, there must be a time component associated with trophic-factor release to avoid what is called growth-factor oasis. Regeneration experiments with gel foam have shown that when the trophic-factor concentration within the bridge is too high, the nerve will grow into the material, but will not grow out. Therefore, there must be either a time-release mechanism that shuts off the delivery of trophic factor, or a release of the factor distally to encourage the nerve cell to continue to grow all the way through the bridge. In the peripheral nervous system, when a nerve regenerates across a gap, it makes functional connections on the distal end. Unfortunately, in the central nervous system, there has not been enough regeneration across the gap to enable prediction of downstream events.

Research in the area of neural development has shown that there are two main principles that influence the elongation of an axon—haptotaxis and chemotaxis. As the growing tip of an axon advances, it detects differential adhesion pathways that guide its course. These adhesive or nonadhesive substrates are generated either by extracellular matrix molecules or are presented on so-called "guidepost" cells on the surface of cell membranes. As the growth cone travels over long distances, it is either attracted or repelled along a certain path. This is haptotaxis. When the axon tip approaches the target cell, the target usually secretes a diffusible factor and the gradient is sensed by the growth cone and it follows the gradient to make the proper connection with the target cell. This is chemotaxis. Hence, it is the combination of the haptotaxis and chemotaxis that enables the growth cone to establish proper connections with appropriate targets.

By reason of the revolution in molecular biology over the past 20 years, the molecular machinery that implements this strategy is largely known. The extracellular matrix molecules that have been implicated include laminin, L1 and NCAM; chondroitin sulfates and other proteoglycans; and hyaluronic acid. The list of neurotrophic growth factors that play a role in regeneration includes, but is not limited to, NGF, BDNF, GDNF, NT-3, and NT-4/5. The question is, Can biomaterials be used to recreate these environments?

The ideal bridge material should be three-dimensional because nerves are three-dimensional. Ideally, its mechanical properties should match those of neural tissue. Tubular polymer guidance channels have been shown to improve regeneration when used as nerve bridge substrates. However, histological analysis shows that the regenerated nerve is always in the center of the tube, with the nerve never touching the sides of the tube. Therefore, the classical approach of modifying the inner

surface of the polymer guidance channel is not likely to be beneficial. Thus, a three-dimensional gel or other soft materials would be good candidates to further enhance regeneration in the peripheral nervous system. Ideally, the gel would have the capacity to have selected adhesion and diffusion cues embedded within it. It would also be beneficial to embed a slow-release delivery system into this matrix for sustained delivery of trophic factors.

A natural biomaterial of choice is hydrogels. These are very hydrophilic chains of polymers that are networked in three dimensions. Previous research from our laboratory has demonstrated that hydrogels are greatly permissive for many types of neurons in that they promote three-dimensional nerve regeneration perpendicular to the plane of culture. We have also determined the optimal porosity and stiffness these hydrogels should have with a mathematical model based on the rate of neurite extension in these materials.

ROLE OF HAPTOTAXIC ELEMENTS

As previously mentioned, there is an assortment of growth-stimulating molecules that could be introduced into a scaffold extracellular matrix. One such molecule is Laminin-1, a 900,000 molecular weight protein that is a potent promoter of neurite extension in many systems. In preliminary studies, the potential of a plain agarose hydrogel to support nerve regeneration was compared to that of agarose containing Laminin-1 covalently coupled in 3-D to the backbone of the gel. The results indicated a much more robust growth of both peripheral and central nerves in the substrate containing Laminin-1. Interestingly, if Laminin-1 is not covalently coupled to the agarose, it does not significantly enhance regeneration beyond the control baseline. We have also reported in the literature that growth in 3-D matrices can be receptor-specific.

To quantify the nature of the nerve regeneration, time-lapse movies were made of the DRG growth-cone-extending processes in the laminin-modified gel. A frame-by-frame analysis of the 2-hour movies was performed and revealed a cyclical nature to the neurite outgrowth. Rather than progressing steadily at a uniform pace, the growth cone exhibited a search, displacement, and rest phase. The laminar region of the tip of the growth cone contains filopodial extensions that it sends out to apparently probe the extracellular matrix. Presumably, after some guidance cue is detected, there is a displacement of the growth cone, after which it rests. These three phases are repeated in a cyclical manner, both in gels that contain laminin and in gels that do not. However, time-lapse video microscopy shows us that the period of the cycle is shorter for agarose-laminin than for plain agarose, although the actual rate of displacement of the growth cone is higher, which might explain why the regeneration is more robust in the modified matrix—because more growth cycles are completed in a given period of time.

USE OF MICROTUBULES FOR SLOW RELEASE OF CHEMOTAXIC ELEMENTS

In order to deliver the chemical-diffusion signal into the gel matrix, technology originally designed for use by the Navy to prevent fouling was adapted. Micron-scale lipid tubules, about 40 μm in length and 0.5 μm in diameter, were loaded with nerve growth factor (NGF) in aqueous conditions. Because of their high aspect ratio and the impermeability of their walls to proteins, sustained release of NGF occurs from the ends of the tubes.

To test the ability of this system to stimulate neurite outgrowth, we designed a three-layered agarose gel system with the control saline-releasing tubules on one side and NGF-releasing tubules on the other side of a gel layer that contained E9 chick dorsal root ganglia. Almost all the processes that the DRGs extended grew from the middle layer toward the NGF layer at 24 hours. It is therefore possible to use these sustained-release vehicles to generate gradients of diffusion factors to direct neurite extension or cell migration.

CELL CULTURE EXPERIMENTS

Although the results of these preliminary studies are promising, the question remains whether such approaches help solve the problem of long-term stability of implanted electrodes. One way to explore this question is to coat electrodes with a gel, or other biomaterial, containing an adhesive component and a slow-release compound. Tissue-culture experiments have demonstrated the ability of several extracellular matrix molecules to support neuron growth. They include type I collagen, laminin-rich matrices, and chitosan.

In vitro experiments were conducted to investigate the response of cortical neurons in culture shown

schematically in **Figure 1** with silicon discs coated with collagen I (**Figure 2**) and silicon discs coated with polylysine, either with or without laminin. Cells from E9 chicken cortical regions were placed onto uncoated and coated silicon wafers (**Figure 3**). The cultures were assayed for their attachment, morphology, and proliferation on coated versus noncoated substrates. The stability of the coatings was also analyzed.

Laminin by itself is not very functional on bare biomaterials because of its poor adhesive properties. Typically, biomaterials are coated with polylysine and then laminin is adsorbed to promote its adhesion. Early

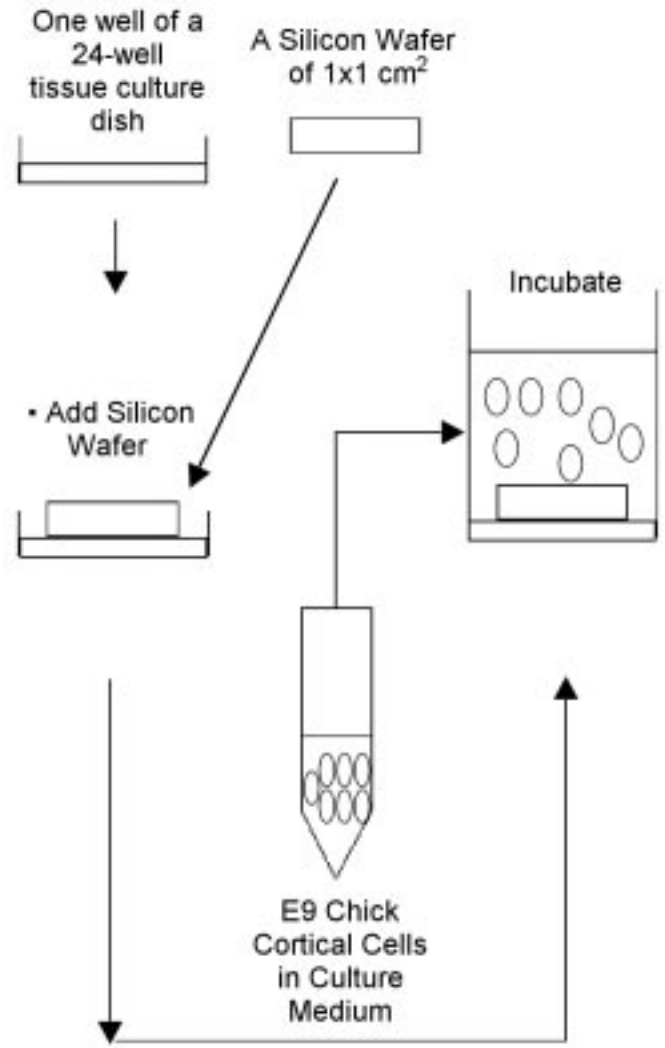

Figure 1.
Diagram representing procedure by which neurons are plated onto silicon. Small silicon wafers are placed into wells of a 24-well polystyrene tissue culture plate. Cortical cells are harvested and placed into wells. After a period of incubation, cell attachment occurs.

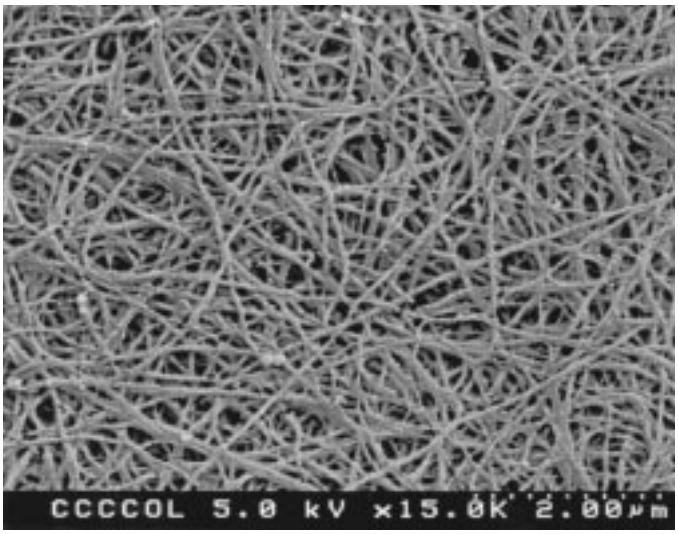

Figure 2.
Electron microscopy reveals presence of a collagen matrix lying on a silicon wafer. Such a positive interaction between collagen and silicon suggests that silicon materials can be manipulated to integrate more favorably with tissue surrounding a silicon-based probe.

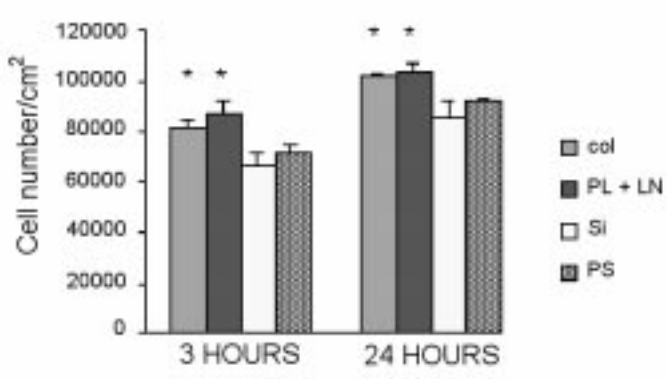

Figure 3.
Attachment of cortical neurons on various coated and uncoated materials was analyzed at 3 and 24 hours. At both time points, more cortical neurons attached to silicon wafers coated with collagen I (col) and polylysine-laminin (PL-LN) than uncoated silicon (Si) or tissue culture polystyrene (PS). Further, statistical analysis performed by conducting a student t-test revealed that higher number of cortical neurons that attached to coated wafers was statistically significant when compared to uncoated silicon ($P < 0.01$).

results in our laboratory indicate that the number of cortical cells attached at the 3-hour and 24-hour time points is significantly increased by the presence of both the collagen and the polylysine-laminin (PL-LN) coatings compared to silicon (Si) or tissue culture polystyrene (PS) (**Figure 3**). However, the increase is modest and there is significant initial attachment on bare silicon also.

When the cell morphology is analyzed at day two, cortical cells do not show much change on collagen, but they do show signs of differentiation on the polylysine-laminin coatings (**Figure 4**). At day three, cortical cells begin to differentiate on the collagen coating and they continue to do so on the laminin (**Figure 5**). Proliferation assays were performed at 1 to 2 days at which time cortical cells appeared to be dividing profusely on both collagen and PL-LN coatings but not on any other substrate. To test the stability of the collagen coating, we incubated coated silicon discs at 37 ºC and stained with a dye for collagen every 3 days. The coating on the discs remained stable for 55 days.

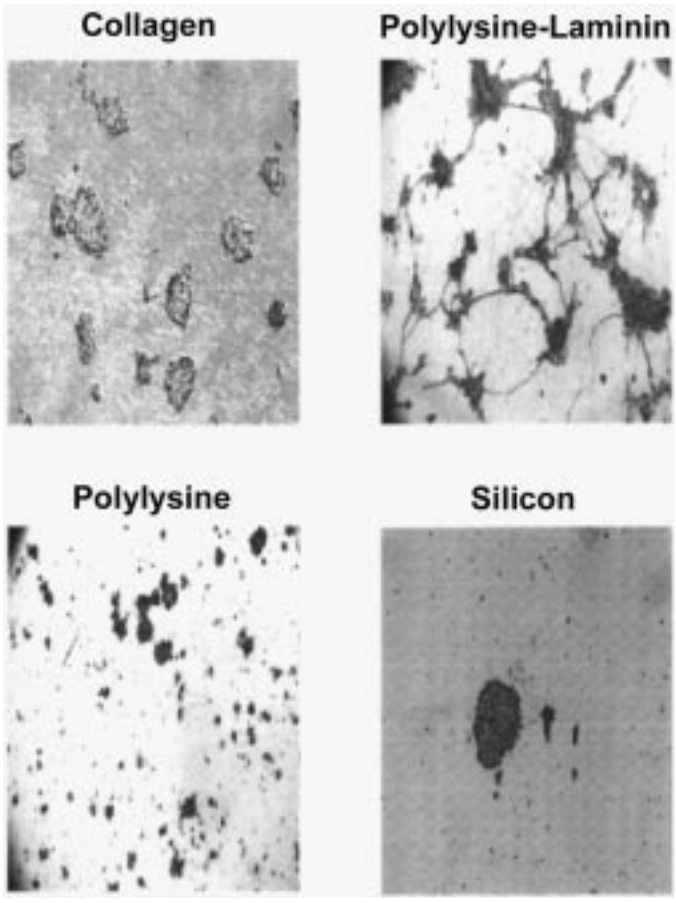

Figure 4.
Cell morphology was examined with bright field microscopy 2 days after cortical cells were introduced to coated and uncoated silicon wafers. Cortical cells on polylysine-laminin-coated silicon wafers had formed interconnections with neighboring cells. These interconnections were not present in cultures containing cortical cells on collagen-coated, polylysine-coated, or uncoated silicon wafers.

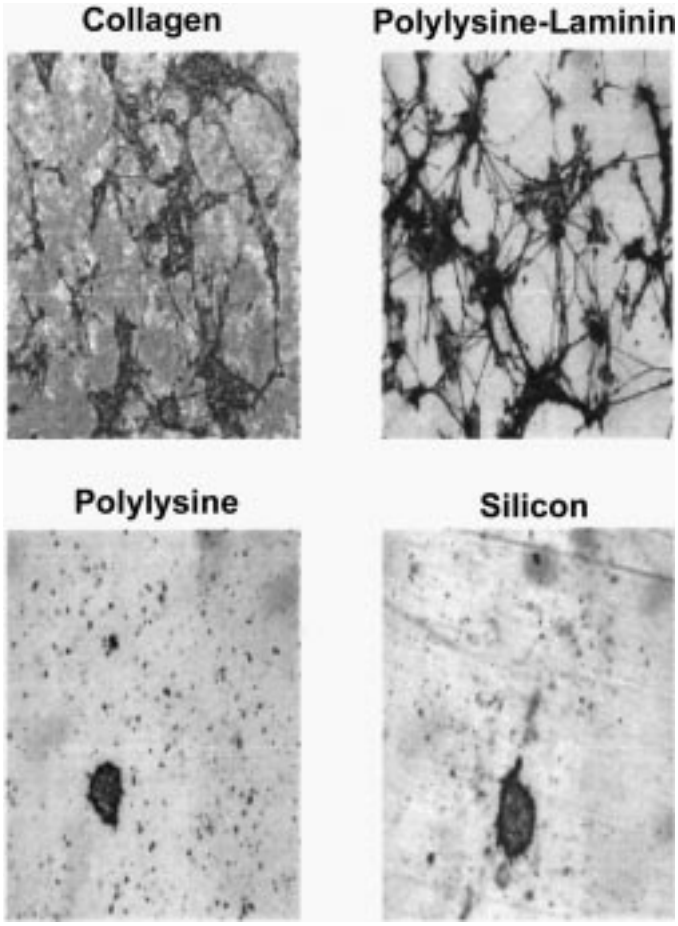

Figure 5.
Cell morphology was examined with bright field microscopy 3 days after cortical cells were introduced to coated and uncoated silicon wafers. As was seen after 2 days of culture, interconnections between cells on polylysine-laminin-coated silicon wafers were still present, with more interconnections present after 3 days than at 2 days. Collagen-coated culture, unlike that which was seen at day 2, started to form interconnections with neighboring cells at day three. Cortical cells on polylysine-coated and uncoated silicon wafers again did not show interconnections with neighboring neurons.

FUTURE RESEARCH DIRECTIONS

Experiments are currently underway to incorporate slow-release components via microtubules into the electrode coatings. Initial data indicate predictable release of proteins for up to 28 days. The duration of release obtained from this tubular system appears to be dependent on two things. One is the concentration of the solution loaded into the tubules, and the other is the length of the tubules, because that affects the diffusion rate. Thus by controlling these two parameters, different periods of release can be achieved for different applications.

Evaluation of longer time periods of collagen stability and of laminin stability is underway. Studies examining the incorporation of microtubules loaded with anti-inflammatory agents and neurotrophic factors into these collagen coatings are also underway.

CONCLUSION

Tissue-engineering strategies are applicable to the development of neurointegrative coatings for implanted electrodes. These coatings can promote cell attachment and differentiation. The coatings can potentially release agents that provide trophic and tropic support to neurons and prevent inflammation locally. Both collagen and polylysine-laminin significantly enhance cortical adhesion and differentiation on silicon substrates as compared to bare silicon or polylysine-coated silicon substrates. Collagen coatings are stable for 55 days when incubated in culture medium at 37 ºC. In addition, it is possible to predictably release proteins from microtubules embedded in the adhesive coatings for up to several weeks.

Therefore, we suggest the field of tissue engineering might provide important insights regarding potential strategies one may adopt to address the problem of electrode-host interface stability in vivo.

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