Protein Adsorption to Materials for Recording Sites on Implantable Microelectrodes

J. Selvakumaran*, M. P. Hughes, J. L. Keddie, D. J. Ewins

Centre for Biomedical Engineering, School of Engineering, University of Surrey,
Guildford, Surrey, GU2 7XH

* current address: Department of Materials, Imperial College London, Exhibition Road,
London SW7 2AZ, UK,

Abstract

The initial adsorption of proteins to materials for implantable microelectrodes is an important factor in determining the longevity and stability of the implant. Implantable microelectrodes have the potential to become part of neural prosthesis to restore lost nerve function after nerve damage. The aim of this study was to identify an optimum material for electrode recording sites on implantable microelectrodes. Common materials for electrode sites are gold, platinum, iridium, and indium tin oxide; these, along with a reference material (titanium) were investigated. Once an implant is in the body, protein adsorption takes place almost instantly before the cells reach the surface of an implant. In this project, the adsorption of proteins to materials for electrode sites has been investigated using atomic force microscopy and ellipsometry.

The protein layers adsorbed to Au after 7 and 28 days were the smoothest and the thinnest compared to all the other substrate materials. Among all the materials studied, Au is the best conductor, the most readily available and has good adhesion strength. Au is the material of choice for electrode recording sites on implantable microelectrodes. This work has demonstrated the suitability of different materials for electrode sites on implantable microelectrodes.

Keywords: microelectrodes, implantable, metals, protein adsorption, recording sites.

1. Introduction

Where permanent nerve damage occurs, the nerves below and above the level of damage generally remain intact and active. Scientists have therefore been trying to overcome the nerve damage by using chronically-implantable microelectrodes to record from and/or stimulate cells of the nervous system (Heiduschka and Thanos, 1998). Partial restoration of lost function has been achieved by advances in functional electrical stimulation (Pechham et al., 1980), however restoration of complex functions, such as standing and walking, require feedback to the stimulator to regulate the output of the stimulator. To achieve this, a number of groups have developed chronically-implantable microelectrodes (e.g. Ensell et al., 1996; Ensell et al., 2000) to interface with the human peripheral nervous system. These types of implantable microelectrodes may also be implanted in the central nervous system as part of neural prostheses; for example a multi-electrode array implanted in the motor cortex and surrounding areas can be used to extract motor signals from the brain, and an online computational device could be used to convert these motor signals into output signals that are capable of controlling an artificial limb (Rousche and Norman, 1998).

Figure 1 shows a schematic of a typical implanable microelectrode probe, consisting of a number of electrode sites located on a penetrating shank that is inserted into a nerve. A mesa structure is used to give strength and to allow interconnection to the outside world. Generally the electrode sites, interconnecting leads, and the bonding pads are made of a conducting metal deposited on a silicon-based substrate. The whole microelectrode is usually insulated by a passivation layer, leaving the electrode sites and bonding pads exposed.

Protein adsorption is believed to be the initial event that takes place when a material is inserted into the body (Andrade and Hlady, 1987; Wahlgren and Arnebrant, 1991). The initially-adsorbed proteins have a significant influence on the subsequent adsorption of further proteins, cells; and microorganisms. They thus dictate the interfacial reactions and ultimately the biocompatibility of the material (Williams et al., 1985). Protein adsorption therefore has a significant impact on the *in vivo* performance of implants.

In the case of electrode recording sites, there should be an intimate contact between the nerve cells and electrodes to maximise the signal to noise ratio. To achieve this, the surface should be covered with a thin and smooth layer of protein as possible. Generally smoother surfaces are preferred to rougher surfaces, because rougher surfaces give rise to higher surface area, thus giving more chance for microbes to adhere and cause infection.

Since adsorption rates vary between different materials, it is necessary to assess the rate at which proteins adsorb to different materials used for recording sites on implantable microelectrodes in order to optimize the performance of the probe. Once in the body, an implant will inevitably be exposed to proteins and ions in the blood and extracellular fluid. Proteins and ions from the extracellular fluid become deposited on the implant first, and then the signals from these adsorbed proteins attract cells to the implant surface. The constituents of diluted blood plasma closely resemble the constituents of extracellular fluid. Therefore protein adsorption studies using diluted blood plasma provide the closest match to the *in vivo* situation.

In this paper, we describe an analysis of protein adsorption from plasma onto materials for electrode sites obtained through the combined use of atomic force microscopy (AFM) and ellipsometry. Common materials used for chronically implantable microelectrodes were chosen for study: gold (Au), platinum (Pt), iridium (Ir), indium tin oxide (ITO) and titanium (Ti). Au, Pt, and Ir were selected because they are the most common materials used for *in vivo* electrode sites (BeMent et al., 1986; Edell, 1986; Drake et al., 1988; Jonzon et al., 1988; Rozman et al., 1988; Anderson et al., 1989; Najafi et al., 1990; Blum et al., 1991; Bradley et al., 1992; Edell et al., 1992; Hoogerwerf and Wise, 1994; Kim and Wise 1996; Kewley et al., 1997; Ensell et al., 2000). ITO was chosen because of its transparency, long-term stability in saline, and its wide use as *in vitro* planar electrodes for recording electrical signals from neurons (Litke and Meister, 1991; Jimbo and Kawana, 1992; Gross et al., 1993; Grattarola et al., 1995; Gopal and Gross, 1996). Although Ti is not a very good conductor, it was chosen as a reference material because it is a long established implant material (Klinger et al., 1998) for which protein adsorption has already been well-characterised (Bess et al., 1999; Hook et al., 2002; Yang et al., 2003). The materials were deposited as thin films (100nm in thickness in most cases) to replicate the thin films used in implantable microelectrodes.

3. Materials and Methods

3.1 Materials

All the materials except ITO were deposited as thin films (100nm) on glass microscope slides (75mm×25mm×1mm). The thickness of the ITO layer is 200nm and it was prepared by “float” process, in which molten glass is floated on a pool of liquid tin under a nitrogen/hydrogen atmosphere (Delta Technologies, MN, USA). Au, Pt and Ti thin films were obtained from the University of Sheffield’s (UK) EPSRC Central Facility for III-V Semiconductors. The Ir slides were obtained from Goodfellow (UK). Au, Pt and Ir had 10nm of Ti to act as an adhesion layer to the glass substrate. Materials were cut to size (10mm×12mm), cleaned with 70% ethanol and then washed three times with distilled water before all the experiments. The backside of the ITO slide used for ellipsometry was roughened using a fine sandpaper to prevent reflection from the back.

3.2 Methods

Rabbit plasma was obtained from Charles River UK and diluted to 1:10 with water to give the final total protein concentration of 5mg/ml. The total protein concentration of the rabbit plasma was determined by using a Pierce BCA protein assay kit (cat # 23225) and Unicam SP 600 series-2 spectrophotometer. A standard curve was constructed by plotting known concentration of bovine serum albumin (BSA) standards against their measured optical density at 562nm. The optical density of the unknown plasma sample was measured and the corresponding concentration was read off from the standards curve. The diluted plasma was preheated to 37°C and centrifuged at 160g for 5 minutes to remove any clumps of protein. The materials were incubated in 8ml of diluted plasma at 37°C for 1day, 7days

and 28 days. At the end of the incubation period, samples were taken out, rinsed briefly with 5 ml of distilled water and dried overnight in a desiccator.

3.2.1 *Ellipsometry*

The ellipsometry was performed on materials exposed to plasma for 1 day to measure the thickness of the protein film. The AFM was used to measure the thickness of protein film adsorbed to materials after 7 days and 28 days exposure to plasma, because the thickness of protein films after these time points were on or above the top range of thickness an ellipsometer can measure.

Ellipsometry does not directly measure the sample parameters of interest such as layer thickness and optical constants; rather it measures the change in polarisation state of light reflected from a surface (Azzam and Bashara, 1977). Two parameters determine the state of polarisation: the amplitude ratio (Ψ) and the phase difference (Δ). The change in amplitude and phase of the polarised light upon reflection is determined in two components: in the plane of reflection (the p-plane) and in the plane perpendicular to the p-plane (the s-plane).

A variable angle of incidence spectroscopic ellipsometer with rotating analyser (J. A. Woollam Co., Inc., Nebraska) was used. The modelling was performed using the WVASE32 software. The interpretation of Δ and Ψ data for an adsorbed protein film into refractive index and thickness of the protein film needs an optical model.

Having obtained values of Ψ and Δ , it is possible to construct a model from which accurate predictions of measurements from a sample of known properties is possible. This model

contains some known parameters such as wavelength of the incident light, polarisation of the incident beam, and the angle of incidence. The model also contains some unknown physical parameters, such as layer thickness and optical constants. It is possible to vary the unknown physical parameters and calculate data until a set of variable parameters, which yields calculated data that very closely matches measured optical data is found. Having found a set of physical parameters that yield calculated data, which closely match the experimental data, it is then important to establish that the set of best-fit parameters are unique, physically reasonable, and not strongly correlated. It is also possible to calculate a number of statistical quantities, which help to evaluate the accuracy and the precision of the fit results.

Scans were performed at wavelengths between 400nm and 800nm in steps of 10nm, and at angles of incidence of 65°, 70° and 75° before and after exposure to plasma for 24 hours. Five replicates of each material were analysed. The values of Δ and Ψ for the bare substrate and the protein layer at different angles of incidence and wavelengths were recorded. Initially Δ and Ψ of the bare substrate were fitted with the corresponding data from the library. Then Δ and Ψ of the protein layer was fitted by building models and changing one parameter at a time to get a best model fit. A Cauchy layer can be used to model materials such as dielectrics, semiconductors, many transparent materials, and organic materials. The index of refraction (n) is represented by a slowly varying function of wavelength (λ):

$$n(\lambda) = An + \frac{Bn}{\lambda^2} + \frac{Cn}{\lambda^4} + \dots \quad \text{Equation 1}$$

A Cauchy dispersion model with two parameters ($A_n = 1.55$ and $B_n = 0.01$ or 0.001) was used to model the protein film. The values of A_n and B_n were varied during the modelling and found that $A_n = 1.55$ and $B_n = 0.01$ fitted proteins on some materials and $A_n = 1.55$ and $B_n = 0.001$ fitted proteins on other materials.

Among all the materials tested only ITO was transparent, and needed two separate layers in the model for glass substrate and the ITO film. For all the other materials the metal layer and the glass substrate were considered as a single layer in the model. The thickness of the glass substrate is 1mm therefore the thickness of the metal layers were set to 1mm in the models for all the materials except for ITO.

3.2.2 Atomic Force Microscopy (AFM)

A NanoScope III (Digital Instruments, California) atomic force microscope was used in this study. AFM was performed on all the materials to obtain topography images of the surfaces before and 1day, 7days and 28days after exposure to plasma. The roughness analysis was performed on all the bare substrates and surfaces exposed to plasma for 7days. The contact mode AFM was used in “window technique” to remove part of the protein film adsorbed to the surfaces before obtaining the topography images using tapping mode AFM. The tapping mode AFM was performed to scan across the steps in “step technique” and section analysis was performed across the steps on surfaces exposed to plasma for 7days and 28days.

At first a technique called “window technique” was used in an attempt to analyse the thickness of protein layer adsorbed to surfaces. In this technique part of the protein layer was removed by the silicon AFM tip in contact mode, a scan was performed across the window at tapping mode, and the vertical distance between the peak and valley (depth) was measured. It was evident from the experience that ordinary silicon tips used to obtain topography images were not strong enough to create windows, as they were broken during the operation and require special diamond AFM tips.

The window technique proved to be difficult with the silicon AFM tip, so there was a need to develop alternative technique to assess the thickness of protein film adsorbed to materials after 7 days and 28 days. A technique was developed to study the thickness of protein film adsorbed to materials after 7 days and 28 days exposure to plasma. In this step technique part of the protein film was carefully removed using cotton buds soaked in trypsin (0.25%) to create a step with sharp edge as possible. Trypsin is an enzyme, which digests peptide bonds in proteins. The debris after trypsin treatment was removed using cotton buds soaked in 70% ethanol and the samples were allowed to air dry for 2 hours. The scans were performed across the step using tapping mode AFM and roughness and section analysis were performed across created step. Fig. 9 shows an example of a topography image across a step. As can be seen the protein on the right hand side of the image has been removed to create a step.

3. Results

3.1 Analysis of bare surfaces

Surface energy and roughness are the two most important properties of the material that determine the adsorption of proteins to surfaces (Horbett et al., 1987). The fabrication process plays a minor role in influencing the surface roughness of a thin-film material. The surface roughness (R_{rms} and R_a) of all the materials are shown in Fig. 2. As the R_{rms} is an indication of the deviation of height from the mean data plane, it gives a better picture of the surface roughness compared to R_a and R_{max} . It can be seen that Ti has the roughest surface and Pt has the smoothest surface. ITO and Ir have similar surface roughness values and they are higher than Pt and Au. The surface roughness of Au is approximately 40% of the surface roughness of Ti, by all three measures of surface roughness. AFM images of all the bare surfaces are shown in Fig. 3-7. It can be seen that Ti has highest amount of surface features (peaks and valleys) and Pt has the lowest amount of surface features. ITO and Ir have similar surface features. From the $1\mu m$ scan of Au and Pt, it can be seen that both of these materials have regular grain structures, but the grains are bigger in the case of Au compared to Pt.

In addition to the above studies, a qualitative assessment of surface free energy was performed (not shown) by depositing $1\mu l$ distilled water onto the surface of the bare substrates and measuring the diameter; the larger the diameter, the more hydrophilic the surface is and the greater the surface free energy. The tests indicated that the surface free energy of Ti and ITO were significantly greater than those of Au, Ir and Pt which were similar to one another.

3.2 Materials Exposed to Plasma for 1Day

The thickness of protein films was obtained from ellipsometry data by measuring Ψ and Δ and building a model to fit the measured Ψ and Δ ; the results for the five materials are shown in Fig. 8 and 9. It can be seen that there are only slight difference between them. ITO has the thinnest layer of protein; this indicates that protein adsorption is minimum on surfaces with medium surface energy. Ti has the thickest layer of protein; this could be due to the hydrophilic nature of the surface. Ti and Ir have a lot of variation on the thickness of protein layer adsorbed to them, indicated by the high standard deviations. The protein layer on ITO is approximately 10 times thinner than on Au and 12 times thinner than on Ti. Tapping mode AFM was performed on all the materials after 1-day incubation with plasma. The topography images are shown in Fig. 3-7. As can be seen, Au and Pt have more uniform surfaces than all the other materials, this correlates with the smooth and regular grain structures seen on the topography images of both bare surfaces. Ir has the highest amount of surface features with deep valleys and high peaks. Although ITO has a high amount of surface features, it can be seen from the contrast in the images that they are not as deep as the surface features seen on Ir or Ti.

3.3 Materials after 7 and 28 days exposure to plasma

At longer exposure times, ellipsometry was found to give poor results due to the thickness of the film, and so tapping mode AFM was used to determine topography and (in window mode) protein thickness on all the materials after 7 and 28 days incubation with plasma. The topography images for 7-day exposure are shown in Fig. 3-7. As can be seen, Au and

Pt have smoother surfaces compared to all the other materials. Ir and Ti have large amounts of surface features compared to all the other materials. ITO has spherical structures scattered across the surface. The thickness of protein layer adsorbed to different materials after 7 days exposure to plasma is shown in Fig. 9. The roughness (R_{rms} and R_a) of protein layer adsorbed to different materials after 7 days exposure to plasma is shown in Fig. 10. Tapping mode AFM was also performed on all the materials after 28 days exposure to plasma. The topography images are shown in Fig. 3-7. As can be seen Ir, Ti and ITO have large amounts of surface features compared to Au. Pt has larger surface features compared to all the materials. The thickness of protein layer adsorbed to different materials after 28 days exposure to plasma is shown in Fig. 9.

4. Discussion

In all cases, the thickness of protein layer adsorbed after 7 days is higher than that of after 1day exposure to plasma, indicating a growth process. The thickness of protein layer adsorbed to Au, Ir, and Pt after 7 days is similar; indicating the rate of adsorption is similar on these three materials up to 7 days, which in turn correlates well with their similar values of surface energy. The rate of protein adsorption onto ITO and Ti is much faster up to 7 days compared to other materials, again correlation well with the surface energy measurements. As can be seen from the topography images, Au and Pt have an apparently continuous layer with uniform thickness after 7 days exposure to plasma, whereas Ti and Ir have peaks and valley structures, and ITO has distinctive spherical structures scattered around the surface. The smoother protein layer on Au could be explained by the lower surface roughness and the rougher protein layer on Ti could be explained by the higher

surface roughness of the bare substrates. This indicates that both the topography and surface energy of the bare substrate have a role in dictating the manner in which protein is adsorbed, but having distinct roles in dictating the protein film thickness and topography respectively. The island formation seen on the ITO slide is dissimilar to all the other sides. After 28 days, the results are more complicated. First, Pt has the highest rate of adsorption after 7 days. The very high thickness value obtained for Pt after 28 days exposure to plasma could be partly due to delamination seen on parts of the Pt sample, some of which is visible in the AFM image, and the results should be examined (and disregarded) in that light. After 28 days exposure to plasma, Au has the smoothest and most continuous layer, ITO has non-uniform features, whereas all the other materials have high peaks and deep valley structures.

Although the mechanisms of the protein adsorption process have been studied and reviewed extensively, many aspects of the mechanisms of the adsorption process remain speculative due to the technical difficulties of the subject (Andrade and Hlady, 1986; Horbett and Brash, 1995). Some of the literature report protein adsorption as irreversible adsorption of protein, but in fact desorption can also occur (Burghardt and Axelrod, 1981; Tilton et al., 1990). For example, Ti has the thickest layer of protein after 7 days and thinnest layer of protein after 28 days, indicating that the rate of protein adsorption is faster than the rate of desorption up to 7 days and the rate of desorption is faster than the rate of adsorption between 7 days and 28 days. Protein adsorption onto Au and Ir follow a slow growth pattern up to 28 days, indicating both adsorption and desorption with rate of adsorption is slightly higher than the rate of desorption. The rate of protein adsorption onto ITO after 7

days is much slower than the rate of adsorption before 7 days. Again this indicates both adsorption and desorption processes with rate of adsorption dominating over the rate of desorption.

From the findings, it is evident that the adsorption of protein to Ti starts with a rapid growth process where the rate of adsorption is greater than the rate of desorption, and reaches an equilibrium point where the rate of adsorption is equal to the rate of desorption and then the rate of desorption becomes greater than the rate of adsorption. The extent of reversibility of protein adsorption depends on the surface energy, surface coverage, and extent of denaturation. Protein adsorption to hydrophilic substrates is generally reversible (Horbett et al., 1987). From the surface energy experiments Ti is the most hydrophilic surface compared to other surfaces and the pattern of protein adsorption on Ti correlates with this. All the other materials are hydrophobic compared to Ti and hence show less irreversible protein adsorption. The exception to this is the case of ITO, but the deposition pattern – consisting of large island structures with little protein deposited between – indicates that the deposition dynamics onto this semiconducting material is quite dissimilar to those of the other materials, and is worthy of further study.

From the results, the material with the roughest and most hydrophilic surface (Ti) adsorbs the thickest layer of protein after 1 day and the thinnest layer of protein after 28 days. The material with the lowest surface energy (Au) adsorbs the thinnest and smoothest layer of protein, and the material with the highest surface energy (Ti) adsorbs the thickest and roughest layer of protein after 7 days exposure to plasma.

5. Conclusions

This work is the first to study and compare the protein adsorption on different materials specifically for microelectrode sites on implantable microelectrodes. In summary, based on the findings reported here and considering other factors such as conductivity, availability, and strength of adhesion to the substrate, it could be concluded that Au is the material of choice for electrode recording sites on implantable microelectrodes. Among all the materials studied, Au is the best conductor, the most readily available and has good adhesion strength. This work has demonstrated the suitability of different materials for electrode sites on implantable microelectrodes.

Acknowledgement

The authors wish to thank the EPSRC for a scholarship for JS. The authors also wish to thank Dr Geoff Hill at the University of Sheffield, EPSRC central facility for III-V semiconductors for thin film preparation, and Dr Peter Zhdan for assistance with the AFM.

References

Alaeddine S., Nygren H. (1995) Logarithmic growth of protein films, in *Proteins at Interfaces II: fundamentals and applications* (Horbett T. A. and Brash J. L. Eds.). Washington, ACS, 41-51.

Anderson D. J., Najafi K., Tanghe S. J., Evans D. A., Levy K. L., Hetke J. F., Xue X., Jappia J. J., Wise K. D. (1989) Batch-fabricated thin-film electrodes for stimulation of the central auditory system. *IEEE Trans. Biomed. Eng.* 36, 693-704.

Andrade J. D., Hlady V. (1986) Protein adsorption and materials biocompatibility – a tutorial review and suggested hypothesis. *Adv. Polym. Sci.* 79, 1-63.

Andrade J. D., Hlady V. (1987) Plasma protein adsorption: the big twelve. *Ann. N. Y. Acad. Sci.* 516, 158-172.

Azzam R. M. A., Bashara N. M. (1977) *Ellipsometry and polarised light*. North-Holland, Amsterdam.

BeMent S. L., Wise K. D., Anderson D. J., Najafi K., Drake K. L. (1986) Solid state electrodes for multichannel multiplexed intracortical neuronal recording. *IEEE Trans. Biomed. Eng.* 33, 230-241.

Bess E., Cavin R., Ma K., Ong J. L. (1999) Protein adsorption and osteoblast responses to heat-treated titanium surfaces. *Implant Dent.* 8, 126-32.

Blum N. A., Carkhuff B. G., Charles H. K., Edwards R. L., Meyer R. A. (1991) Multisite microprobes for neural recordings. *IEEE Trans. Biomed. Eng.* 38, 68-74.

Bradley R. M., Smoke R. H., Akin T., Najafi K. (1992) Functional regeneration of glossopharyngeal nerve through micromachined sieve electrode array. *Brain Research* 594, 84-90.

Burghardt T. P., Axelrod D. (1981) Total internal reflection-fluorescence photobleaching recovery study of serum-albumin adsorption dynamics. *Biophys. J.* 33, 455-467.

Drake K. L., Wise K. D., Farraye J., Anderson D. J., BeMent S. L. (1988) Performance of planner multisite microprobes in recording extracellular single-unit intracortical activity. *IEEE Trans. Biomed. Eng.* 35, 719-732.

Edell D. J. (1986) A peripheral nerve information transducer for amputees: long-term multichannel recordings from rabbit peripheral nerves. *IEEE Trans. Biomed. Eng.* 33, 203-214.

Edell D. J., Vo Van Toi, McNeil V. M., Clark L. D. (1992) Factors influencing the biocompatibility of insertable silicon microshafts in cerebral cortex. *IEEE Trans. Biomed. Eng.* 39, 635-643.

Ensell, G., Banks D. J., Ewins D. J., Balachandran W., Richards P. R. (1996). Silicon-based microelectrodes for neurophysiology fabricated using a gold metallization nitride passivation system. *J. Microelectromech. Sys.* 5, 117-121.

Ensell G., Banks D. J., Richards P. R., Balachandran W., Ewins D. J. (2000) Silicon-based microelectrodes for neurophysiology, micromachined from silicon-on-insulator wafers. *Med. Biol. Eng. Comp.* 38, 175-179.

Gopal K. V., Gross G. W. (1996) Auditory cortical neurones in vitro: cell culture and multichannel extracellular recording. *Acta Oto-Laryngologica* 116, 690-696.

Grattarola M., Bove M., Verreschi G., Martinola S., Tedesco M., Chiarugi S. (1995) The engineered petri dish – a new tool for biotechnology. *Minerva Biotecnologica* 7, 228-233.

Gross G. W., Rhoades B. K., Reust D. L., Schwalm F. U. (1993) Stimulation of monolayers networks in culture through thin-film indium tin oxide recording electrodes. *J. Neurosci. Meth.* 50, 131-143.

Heiduschka P., Thanos S. (1998) Implantable bioelectronic interfaces for lost nerve functions. *Prog. Neurobio.* 55, 433-461.

Hoogerwerf A. C., Wise K. D. (1994) A three dimensional microelectrode array for chronic neural recording. *IEEE Trans. Biomed. Eng.* 41, 1136-1146.

Höök F., Vörös J., Rodahl M., Kurrat R., Böni P., Ramsden J. J., Textor M., Spencer N D., Tengvall P., Gold J., Kasemo B. (2002) A Comparative Study of Protein Adsorption on Titanium Oxide Surfaces using in situ Ellipsometry, Optical Waveguide Lightmode Spectroscopy, and Quartz Crystal Microbalance/Dissipation. *Colloids and Surfaces B: Biointerfaces.* 24, 155–170.

Horbett T. A., Brash J. L. in Brash J. L., Horbett T. A. Eds. (1987) *Proteins at Interfaces: physicochemical and biochemical studies.* ACS, Washington, DC, pp1-36.

Horbett T. A., Brash J. L. (1995) *Proteins at Interfaces II: fundamentals and applications.* ACS symposium series 602, Washington, DC.

Jimbo Y., Kawana A. (1992) Electrical stimulation and recording from cultured neurones using a planar electrode array. *Bioelectrochemistry and Bioenergetics* 29, 193-204.

Jonzon A., Larsson E. N., Oberg P.A., Sedin G. (1988). Long-term implantation of platinum-electrodes - effects on electrode material and nerve-tissue. Med. Biol. Eng. Comp. 26, 624-627.

Kewley D. T., Hills M. D., Borkholder D. A., Opris I. E., Maluf N. I., Storment C. W., Bower J. M., Kovacs G. T. A. (1997) Plasma etched neural probes. Sens. Actuat. A. 58, 27-35.

Kim C., Wise K. D. (1996) A 64-site multishank CMOS low profile neural stimulating probe. IEEE J. Solid-State Circuits 31, 1230-1238.

Klinger M. M., Rahemtulla F., Prince C. W., Lucas L. C., Lemons J. E. (1998). Proteoglycans at the bone-implant interface. Crit. Rev. Oral Biol. Med. 9, 449-463.

Litke A., Meister M. (1991) The retinal readout array. Nuclear Instrum. Meth. Phys. Res. A- Accelerators Spectrometers Detectors and Associated Equipment 310, 389-394.

Morris G. et al. (1999) Long-term follow-up on 454 patients receiving vagus nerve stimulation therapy. Neurology 53, 1731-1739.

Najafi K., Ji J., Wise K. D. (1990) Scaling limitations of silicon multichannel recording probes. IEEE Trans. Biomed. Eng. 37, 1-10.

Norde W., Anusiem A. C. I. (1992) Adsorption, desorption and readsorption of proteins on solid-surfaces. *colloids surfaces* 66, 73-80.

Norde W., Haynes C. A. (1995) Reversibility and the mechanism of protein adsorption, in *Proteins at Interfaces II: fundamentals and applications* (Horbett T. A. and Brash J. L. Eds.). Washington, ACS, 26-40.

Pechham P.H., Mortimer J.T., Marsolais E.B. (1980) Controlled prehension and release in C5 quadriplegic elicited by functional electrical stimulation of the paralysed forearm musculature. *Ann. Biomed. Eng.* 8, 369-388.

Rousche P.J., Norman R.A. (1998) Chronic recording capability of the Utah intracortical electrode array in cat sensory cortex. *J. Neurosci. Meth.* 82, 1-15.

Rozman J., Pihlar B., Strojnik P. (1988). Surface examination of electrodes of removed implants. *Scand. J. Rehab. Med. Suppl.* 17, 99-103.

Tilton R. D., Robertson C. R., Gast A. P. (1990) Lateral diffusion of bovine serum-albumin adsorbed at the solid liquid interface. *J. Colloid Interf. Sci.* 137, 192-203.

Wahlgren M., Arnebrant T. (1991) Protein adsorption to solid surfaces. *Tibtech.* 9, 201-208.

Williams D.F., Askill I.N., Smith R. (1985) Protein adsorption and desorption phenomena on clean metal surfaces. *J. Biomed. Mater. Res.* 19, 313-320.

Yang Y., Cavin R., Ong J. L. (2003) Protein adsorption on titanium surfaces and their effect on osteoblast attachment. *J Biomed. Mater. Res.* 67A, 344-9.

Figure captions

- 1: Pictures of a typical neuroprobe. Left: a schematic diagram (not to scale) of a typical neuroprobe showing the different components. Right: a scanning electron micrograph of one of the probes showing the shank with electrode recording sites.
- 2: The surface roughness (R_{rms} and R_a) of different materials. The values obtained from a $9\mu\text{m}^2$ area scan for each material.
- 3: Topography images of Au. a) bare substrate, and b) 1 day, c) 7 days, and d) 28 days exposure to plasma.
- 4: Topography images of Ir. a) bare substrate, and b) 1 day, c) 7 days, and d) 28 days exposure to plasma.
- 5: Topography images of ITO. a) bare substrate, and b) 1 day, c) 7 days, and d) 28 days exposure to plasma.
- 6: Topography images of Pt. a) bare substrate, and b) 1 day, c) 7 days, and d) 28 days exposure to plasma.
- 7: Topography images of Ti. a) bare substrate, and b) 1 day, c) 7 days, and d) 28 days exposure to plasma.
- 8: Thickness of protein layer on different materials after exposure to plasma for 1 day.
- 9: Thickness of protein layer adsorbed to different materials after 1, 7, and 28 days exposure to plasma.
- 10: Surface roughness (R_{rms}) of bare substrate, protein layer adsorbed to different material after 7 and 28 days exposure to plasma.

Figures

Fig. 1.

Fig 2

Fig. 3:

Au

Fig. 4:
Ir

Fig. 5:
ITO

Fig. 6:
Pt

Fig. 7:
Ti

Fig. 8:

Fig. 9:

Fig. 10: