DLC based BioMEMS probe for electrical activity recording of tissues and cells

Carmen Moldovan\textsuperscript{a}, Rodica Iosub\textsuperscript{a}, B. Firtat\textsuperscript{a}, Claudia Roman\textsuperscript{a}, C.P. Lungu\textsuperscript{b}, Ana Lungu\textsuperscript{b}, R. Albulescu\textsuperscript{c}

\textsuperscript{a} National Institute for R&D in Microtechnologies (IMT-Bucharest)
\textsuperscript{b} National Institute for Laser, Plasma and Radiation Physics, Magurele
\textsuperscript{c} National Institute for Chemical-Pharmaceutical, Bucharest
GOAL

Recording of the neuronal electrical activity for developing an useful tool for biomedical applications, in studying the neural mechanisms underlying cognition
INTRODUCTION

DESIGN AND FABRICATION

DLC DEPOSITION BY THERMIONIC VACUUM ARC (TVA)

BIOCOMBATIBILITY tests

CONCLUSIONS
INTRODUCTION

Two sorts of neuronal activity could be studied using microelectrode recordings:
- the local field potential which mainly reflects synaptic activity and
- the spiking activity which reflects the neuronal output - the signal that is sent to other neurons.

The low signal to noise ratio and high impedance signal transfer are important problems to be solved.

- An implantable probe fabricated on a silicon substrate for electrical activity monitoring of living tissues was developed and fabricated.
- In order to improve the mechanical resistance and biocompatibility of the device, the technology of Thermionic Vacuum Arc (TVA) deposition was used for coating the implantable parts with diamond like carbon (DLC) with zero stress (0SC), at the end of silicon processing steps.
**MAIN ACHIEVEMENTS**

- **Design and manufacturing** steps of an DLC based 8-channel microprobe for recording the electrical activity of neural cells and tissues.
- The electronics implemented on the board accomplish the separation and reduction of the biological noise recording.

  **Testing functionality and biocompatibility**
  - The microprobe functionality was tested in vivo and in vitro, in specialized laboratories, by recording electrical signals from cells cultures and mice organs. Biocompatibility tests were performed on implantable microprobes, coated with DLC/0SC, introduced in cells cultures.

- **Applications**
  - The integrated microprobe for monitoring tissues electrical activity can be used in laboratories and research centres acting in the biomedical field, which study the cells growth and their response to physico-chemical stimuli, in hospitals and treatment centres for people suffering from neurological diseases.
The technologies for MEMS fabrication are mainly based on silicon (Si), but Si exhibits poor tribological properties for MEMS applications (low mechanical resistance, high friction) and reduced biocompatibility.

In order to improve the mechanical resistance and biocompatibility of the device, the implantable parts were coated with diamond like carbon (DLC) films with near zero stress.

Physical properties of **DLC layers:**
- low friction coefficient
- increased hardness
- thermal and mechanical stability
- chemical inertness, infrared transparency, high electrical resistivity

The DLC layers were deposited using the **Thermionic Vacuum Arch method.**
DESIGN AND FABRICATION

- CHARACTERISTICS
- SIMULATION
- DESIGN
- FABRICATION
CHARACTERISTICS of the microprobe:

The microprobe has a thin tip of 3-10 mm length (3 mm for the human cells implant and 10 mm for the rat’s cell implant respectively).

The tip width is:
- 30 µm for 4 channels microprobe
- 60 µm for 8 channels microprobe
- 10 µm for the microprobe with neural insertion

The tip thickness is 20 µm for neural insertion probe and 100 µm for muscular insertion probe.

The microprobe has a 3x4 mm² surface which serves as support for manipulation and electronics.
Simulation of a microprobe tip covered with DLC film helped us establishing the microprobe design.

The simulation was realized in COVENTOR programme for a microprobe with 3 mm length of the tip and 20 µm thickness and a constant pressure of 10 MPa.
LAYOUT

The recording/stimulation microprobe can be realized by processes of **bulk micromachining**: laser machining, double side alignment, metal deposition, layers patterning. The anisotropic etching of silicon was studied for obtaining a microprobe with forms and dimensions precisely controlled and a well defined tip.

The signals collected from the microprobe need to be amplified and processed in order to obtain useful information. For this reason, an interface between the microprobe and the laboratory equipment must be realized. The implementation of this interface was done using hybrid technology with discrete components.
MANUFACTURING STEPS

a. Oxidation + $\text{Si}_3\text{N}_4$ deposition
   Ion implantation + diffusion
   Polysilicon 4000 Å deposition

b. TiW/Au deposition

c. 1 $\mu$m CVD deposition

c. Microprobe separation by etching in EDP
   DLC deposition
DLC FILMS DEPOSITION by TVA method (Thermionic Vacuum Arc)

- DLC DEPOSITION
- DLC CHARACTERISATION
Diamondlike carbon (DLC) is a metastable material:
- amorphous carbon, a-C
- hydrogenated amorphous carbon, a-C:H: contains from < 10% to 60% hydrogen. Incorporation of hydrogen in this type of DLC is important for obtaining diamondlike properties.

The thermionic vacuum arc (TVA) discharge with evaporating anodes employs directly heated thermionic cathodes. The TVA discharge generates a pure, gas-free metal vapor plasma. TVA is strongly controlled by the cathodic electron beam and there is a quite good stability of important operation parameters like the arc voltage and the arc current.

Because this system allows the carbon evaporation, it is one of the most adequate technology for obtaining hydrogen free diamond like carbon layers.

We deposited DLC films from graphite bars of 10 mm diameter. The substrates were not heated in advance; the temperature during the deposition process was around 100-300°C, only due to the TVA radiation and ion sputtering.
The DLC films were investigated using **HRTEM** (High Resolution Transmission Electron Microscopy) and **SAED** (Selected Area Electron Diffraction) methods.

From HRTEM analysis, interference beams could be observed, given by the nanostructured particles of diamond and graphite from the amorphous carbon film. The arrows show the interplanar distances corresponding to crystalline structures.

By analysing the diffraction pattern with SAED technique, rhombohedral structures were identified, with lattice parameters: \(a = 0.25221\) nm and \(c = 4.3245\) nm (*ASTM pattern: 79-1473*), corresponding to diamond.

The films were adherent to the Si substrate and determined improved mechanical properties (especially the fracture toughness) of the Si tips.
MICROPROBE CHARACTERISATION

SEM picture of the microprobe tip after 5 h etching in EDP, 96°C

Optical picture of the microprobe and bonding pads (x300), after 5 h etching in EDP at 96°C

Optical picture of the released microprobe tip (x300)
For testing the functionality, the microprobe was packed using gold wires bonding on a copper board, in order to allow the electrical signals reading and processing. The electronics accomplish the separation and reduction of the biological noise recording.

Packaging in “pen” shape allows the device handling in biological environments, respectively insertion in small quantities of liquids, or cell culture.
The microprobe functionality was tested in vivo and in vitro, in specialized laboratories, by recording electrical signals from cells cultures and mice organs.

The impedance measurements revealed different values for different tissues and organs, but reproducible at the same tissue/organ level.

Biocompatibility tests were performed on implantable microprobes, coated with zero stress DLC, introduced in cells cultures.

The standard procedure was based on citotoxicity tests in vitro, using fibroblasts cells L929. The cells viability was estimated by functionality (evaluation of cells breath, protein synthesis, DNA quantification) and permeability tests.
An improvement of cells adhesion and growth was observed for microprobes coated with DLC films.

The extracting and contact methods proved that no significant differences exist between the viability of the treated environment and the control one, therefore no citotoxic products from the tested materials are released into the growing cell environment.
CONCLUSIONS (1)

- An implantable neural microprobe was developed in order to enable the correlation between electrical activity in the central nervous system and externally psychoelectrical stimuli to be investigated.
- The microprobes based on carbon materials were functional from the electrical and mechanical point of view.
- This microprobe has the advantage of silicon processing combined with the use of new biocompatible materials with improved mechanical resistance – diamond like carbon films.
Coating the Si based microprobe with DLC films proved the utility of using TVA technique for improving the mechanical properties and biocompatibility of the microprobes. The low temperature of TVA method allows the deposition of DLC after the metallic layers are configured.

The tests performed on the obtained Si microprobes coated with DLC films showed an improvement of cells adhesion and growth and no citotoxic response for the implantable device.

The development of new manufacturing processes and materials for MEMS in Biomedical applications is leading to multifunctional devices with low power, reduced mass, minimum cost, high functioning degree at macro and micro scale.
THANK YOU!

cmoldovan@imt.ro