Evaluation of Dispensed Carbon Nanotube Ink on Flexible Substrates for Biocompatible Application

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Abstract

For biomedical electronics the compatibility to the biological environment should be well-considered. Therefore this paper evaluates dispensed carbon nanotubes (CNT's) on polyimide (PI) foil for conductive tracks and electrodes for flexible, biomedical applications. A CNT based ink is investigated regarding biocompatibility, flexibility, conductivity and suitability for electrode materials with contact to artificial body fluids. The testing methods comprise bending tests with resistance monitoring, adhesion tests and the utilization of dynamic fluidic and electrical load on dispensed structures. The CNT ink showed good bending properties up to 2653 cycles with an average sheet resistance of 32.5 Ohm/sq. A demonstration of biocompatibility using the adherent cell line HFFF2 resulted negatively. No delamination or dissolving effects occurred during exposure to 0.9 % sodium chloride solution.

Motivation

For the development of flexible biocompatible electronics, individual but reliable packaging is still one of the key challenges. Especially materials for devices with tissue contact, such as electrodes for skin current stimulation, electrochemotherapy, nerve electrodes or encapsulations of implantable microsystems need to be chosen in distinct awareness of their possible interaction with surrounding biological matter. Firstly they must not harm the biological environment by dissolving unknown components. Secondly they as well must not show any instable behavior and loose functionality prematurely due to harsh ionic environment. The state of the art materials for electrodes and conductive tracks for biomedical applications are precious metals such as gold [1], platinum [2] and titanium [3]. But also carbon, lately often in form of carbon nano tubes (CNT) has been proofed as valuable alternative, due to its excellent mechanical, electrical and chemical properties [4-6].

This paper evaluates the suitability of additively processed carbon nanotube (CNT) ink for conductive tracks and electrode material for biomedical application. Due to the favorable electrical properties of CNT's, applications for conductive tracks [7], transistors [8] and radio-frequency identification (RFID) tags have already been published [5]. CNT's for biomedical applications, e.g. for biological sensing has been a major field of research [4,5]. But the biocompatibility of CNT materials is controversially discussed in literature [5]. On the one hand cell tests with L929 mouse fibroblasts and Human Umbilical Vein Endothelial Cells (HUVEC) cell lines stated good results regarding cell growth [9] on CNT materials. But on the other hand cells exposed to CNT's

showed inflammatory reactions, resulting in DNA and cell damage [10, 11]. For flexible, biocompatible packaging of electronics the cytotoxicity has to be assured according to standard ISO 10993-1 for the biological evaluation of medical devices. In relation to this, the way of fabrication has to be considered as well. Since most of established subtractive processes in electronics packaging such as etching or lift-off after PVD processes deal with highly toxic chemicals, which may remain after the fabrication process within the microsystem. That issue can be overcome by using additive processes applying non-toxic materials only on required positions. Therefore CNT materials are considered a low cost and low temperature additively processing alternative to metal. Furthermore the fabrication processes for applying CNT's on substrates such as dip and spray coating, screen-, aerosol-, transfer- and contact printing [4, 12] are especially promising for biocompatible electronics. Yet every process requires specific material properties e.g. viscosity, homogeneous layers or surface tension. Therefore printable materials such as inks and pasts will be chemically functionalized which will lead to the favorable properties. Therefore the biocompatibility, flexibility and adhesion will depend on the formulation of additively processing materials and has to be evaluated before using for biomedical applications.

Materials and Methods

This paper describes an approach to evaluate dispensed aqueous carbon nanotube ink for conductive tracks on substrates from biocompatible flexible substrates, through a series of test methods. The used CNT-Ink consists of 0.1 wt% single wall carbon nanotubes (SWCNT) dispersed in water. Other additives are named as non-hazardous hydrocarbons, water soluble salts and transition metals. Exposure of the CNT ink to reconstituted human epidermis for 24 h showed no sign of toxicity according to MSDS.

Figure 1. SEM image of cured CNT ink

The manufacturer recommends curing for 10 min on a hotplate at 100 °C. Figure 1 shows the surface of cured

CNT ink with random alignment of the CNT's. The tests in this paper were carried out on polyimide PI-2611 substrates (HD Microsystems), which is typically used for biomedical applications [2, 13-15]. The polyimide has been spun on Si wafer to achieve a uniform surface with an average thickness of 9.5 μm after curing. Dispensing of the CNT ink has been done using a needle with 60 μm inner nozzle diameter. The wetting of the ink could be enhanced by ozone treatment as shown in Table 1. After good wetting, the substrate was placed on a hot plate for curing, which led to immediate contraction of the drop. This effect did not occur without ozone treatment. However forming tracks with (100, 200, 300) μm nominal line widths, only was possible using a laser drilled ceramic mask for local ozone treatment and self-alignment of the ink (Figure 2).

Table 1. Contact angle before and after 10 min ozone treatment

Test medium	Contact angle before in grad	Contact angle after in grad
CNT ink	40.4	3.5
Deionized water	66.6	11.0

Figure 2. Cured dispensed and self-aligned CNT ink with 100 μm line width

After dispensing the CNT ink with an average layer height of $(1.1 \pm 0.05) \mu\text{m}$, the achieved layer width and the electrical resistance were determined (Table 2). The average sheet resistance is about 32.5 Ohm/sq. Contacting CNT ink electrically revealed as challenging tasks. Since standard processes like soldering, sintering and bonding are unsuitable, the ink was contacted using test probes or conductive adhesive.

Test on Cytotoxicity

For biomedical application with direct contact to tissue a test on cytotoxicity has to be carried out according to standard ISO 10993-5. Therefore a human fibroblast adherent cell line HFFF2 from Sigma-Aldrich Chemie GmbH was chosen. For sample preparation PI 6211 was spin coated and CNT ink with different solvents (Water, Ethanol, Methyl Ethyl Kethone (MEK)) was dispensed on biocompatible glass slides, cured and placed in a multiwell plate. After 30 min of UV sterilization the samples were incubated with 150.000 cells in Dulbecco's Modified Eagle's Minimum Essential Medium (DMEM) mixed with Ham's F12 in ratio 1:2 (DMEM/F12) and supplemented with 10% fetal calf serum (FCS). The in-

Table 2. Line widths and electrical resistance of line structures with 72 mm length

Nominal line width in μm	Measured line width $\pm$ standard deviation in μm	Mean electrical resistance $\pm$ standard deviation in Ohm
100	119.2 ± 9.6	21375.6 ± 412.5
200	212.1 ± 7.6	10879.4 ± 501.1
300	311.7 ± 12.4	6369.4 ± 399.7

cubation endured 48 h at 37 $^{\circ}\text{C}$ under CO_2 (5 %) atmosphere. For cytotoxicity evaluation the CellTiter-Blue® (CTB) Cell Viability Assay of Promega GmbH was added to the multiwell plate. It contains the blue indicator dye resazurin, which is used to measure the metabolic capacity of cells. Only viable cells are able to metabolize resazurin into the pink dye resorufin. The quantity of resorufin produced, is proportional to the number of viable cells. Hence a light absorbance measurement at 560 nm and 590 nm wavelength after 5 h additional incubation with CTB assay indicates the quantity of viable cells grown on the CNT material.

Bending Tests

Conductive tracks for flexible applications need to be tested for reliability while bending or stretching. Therefore a test setup for bending and simultaneously resistance monitoring of flexible substrates for electronics was used, which has been previously published [16,17]. The CNT ink was dispensed in U-shaped structures of (100, 200, 300) μm nominal widths and up to 3 layers for the 300 μm line. The PI foil was removed from the wafer and attached to the test setup using a 5 mm bending diameter and 2700 cycles. For resistance monitoring and failure detection, the ink was contacted with Ag conductive adhesive H20E (Epoxy Technology Inc.). Since H20E becomes rigid after curing, the contact itself should be obviated from bending.

Tests under fluidic load

For biomedical applications with contact to body fluids a test of CNT material in 0.9 % sodium chloride solution was performed to investigate delamination or dissolving issues. Therefore circle shaped structures (10 μl ink) on PI are cured and placed in a fluid chamber, which provides a laminar fluid dynamic load [18]. At the same time structures have been placed in a beaker for static fluidic load and examined using microscopy afterwards. The test parameters are shown in Table 2. Furthermore a test with static fluidic and electrical load has been performed with a CNT cathode and a graphite anode in 0.9 % sodium chloride solution. Therefore CNT ink has been dispensed on PI which is attached to a ceramic substrate. The contact pad and the track were covered by isolating PDMS. Figure 3 shows the setup without contacting wires of the electrodes and measuring equipment. The distance between the electrodes was 40 mm. A voltage of 1 V DC was applied for 24 h to examine possible reliability issues for short term medical application. The voltage was chosen below the theoretical

value of about 1,23 V without considered overpotential for hydrogen production through electrolysis of sodium chloride solution [20]. The test parameters are shown in Table 3.

Table 3. Parameter for test under fluidic load in 0.9 % NaCl solution

Parameter	Dynamic fluidic load	Static fluidic load	Electric static fluidic
Duration	30 d	30 d	24 h
Temperature	37 °C	37 °C	37 °C
Flow velocity	0.14 m/s	-	-
Voltage DC	-	-	1 V

Figure 3. Test setup for electrical load on dispensed CNT electrodes in NaCl solution

Adhesion test

Furthermore an adhesion test was performed according to the standard ISO 2409. Therefore a right angle lattice pattern with 6 cuts in each direction and 1 mm distance of each was cut in a layer of cured CNT ink. Adhesion test was performed with the tapes Tesapack 4124 PVC and Scotchtape 508. The quality of adhesion can be characterized between 0 for good adhesion and 5 for no adhesion.

Results

Test on Cytotoxicity

Figure 4. Results on cytotoxicity in relation to a 100 % viable reference

Figure 4 shows the results of the CTB viability test. CNT ink and the PI substrate are depicted in relation to a biocompatible glass reference, representing 100 % cell viability. PI shows a similar cell growth as the biocompatible glass reference. The CNT in different solvents show 30 % to 60 % viability with the used cell line.

Bending tests

Figure 4 shows the linear fit of the monitored resistance of the U-shaped structures during bending tests. No significant increase of the resistance during the testing period can be seen. None of the tested structures reached the maximum cycle count of 2700. The structure with 100 μm in average attained the highest cycle count of 2653. The results in Figure 5 show the more layers are dispensed and hence the thicker the structures get, the earlier failures while bending occur.

Figure 5. Linear fit of the resistance during bending cycles until failure of the structure

Figure 6. Attained bending cycles of 300 μm lines with different layer counts before failure. The error bars indicate the maximum and minimum number of cycles reached of all tested structures

Tests under fluidic load

The result of fluidic dynamic and static load showed similar results – no change or delamination after 30 days of testing can be seen with microscopy (Figure 7).

Figure 7. CNT structures a) before and b) after 30 days of fluidic dynamic load

The results after electrical and fluidic load showed no delamination or dissolving effect evaluated with light microscopy. Figure 8 shows the current monitored during the experiment for 24 h. At the beginning a decreasing current because of the double-layer formation in electrolytes can be seen. However a slight decrease during the test period indicates an alteration of material since the voltage is below electrolysis level, which alters the fluid.

Figure 8. Monitored current during electrical and fluidic load

Adhesion test

The adhesion test was performed five times (test 1) and repeated after 2 month (test 2) for another 5 times. Both tapes showed similar results. During test 1, not more than 5 % of the lattice of the CNT ink was pulled off the PI substrate. Whereas the ink showed no adhesion at all during the second test (Figure 9). Those results imply altering properties of the ink during two month of storage. Also a higher part of agglomerated CNT particles forming larger particles were noticed after using the ink for the second test.

Figure 9. Tape test showing altering adhesive properties over time

Conclusions

This paper evaluates dispensing of CNT ink for flexible biocompatible applications. Dispensing using a 60 μm nozzle is eligible for conductive tracks dimensions larger than 100 μm due to high drop volumes. Smaller feature sizes can be reached by inkjet or aerosoljet printing, however nozzle clogging caused by linked up CNT's will be an issue.

Bending tests showed that one layer of CNT ink for flexible applications attains highest cycle counts without failure. However the relatively high resistance in kilo ohm range should be considered for future applications.

CNT ink in 0.9 % sodium chloride solution for electrodes with contact to body fluids does not cause delamination or dissolving issues analysed in this paper. However, the test with electrical and fluidic load indicates an alteration of material. Chemical, XPS or scanning microscope investigations should be considered to further investigate the CNT properties in electrolytic medium.

The adhesion test shows contradictory results indicating altering properties of the ink during 2 month. This suggests an awareness of possible aging behaviour of the ink for usage for a longer period, which should be further looked into.

The cytotoxicity test with the adherent cell line HFFF2 showed a toxic impact on the cells not dependent on solvents. However no absolute statement about the cytotoxicity and the biocompatibility can be made and the influence of additives on the results has to be investigated. Further tests with different cell lines should be done. For biocompatible application this CNT ink can be used with a biocompatible flexible encapsulation.

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