NON-PLANAR ELECTRODES MADE FROM BOND WIRES FOR IMPEDANCE SPECTROSCOPY OF 3D-MICROTISSUES

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ABSTRACT

We present a new versatile method for integration of out-of-plane microelectrode configurations on planar microchips. The technology relies on the precise formation of user-defined gold bond-wire loops and has been used to develop a multi-electrode electrical microimpedance device for interrogating 3-dimensional microtissue structures. 3D microtissue spheroids are receiving growing attention, as they closely reproduce *in vivo* conditions in drug screening applications.

BACKGROUND / STATE OF THE ART

Microtissue spheroids constitute a popular three-dimensional tissue-model system in pharmaceutical compound development and are widely used as organ models in basic research [1]. Owing to their increased use over the past years, new readout methods are required that are compatible with these three-dimensional microtissue structures. Most analysis methods are applied to cell-based assays and are optimized for 2D cell layers. This holds also true for electrical impedance spectroscopy (EIS), a label-free analysis method, which holds great potential for studying cell and tissue structures [2], but which mostly relies on planar microelectrode configurations. Devising 3D microelectrode configurations that are amenable to investigate spheroid microtissue structures is challenging, as most microfabrication processes rely on planar photolithographic patterning methods.

DESCRIPTION OF THE NEW METHOD OR SYSTEM

In this paper we introduce a fabrication concept for vertical microelectrode configurations through a versatile process and demonstrate their application in electrical impedance measurements. The three-dimensional electrode configurations were formed from gold wires by using a ball-wedge automatic wire-bonding process. By varying the loop shaping parameters, not only the height of the electrodes, but also the inclination of the wire loops can be controlled.

The micro-impedance configuration was fabricated by arranging eight of these microelectrodes in a circular pattern around a micro-well. A 100 mm / four inch glass wafer (Borofloat33) was used as a substrate. First a two-layer photo resist was spun on and processed to form the lift-off masking layer. Subsequently, a metal stack consisting of a 20 nm WTi10 alloy adhesion layer and a 200 nm platinum layer was deposited by ion beam deposition. With the final lift-off solvent soak and cleaning steps the metal patterns were unveiled, which form the bond pads for the out-of-plane electrodes, a set of coplanar electrodes for comparison and the interconnect contact pads. Thereafter, the wafer comprising four chips with dimensions of 32 mm x 32 mm each was diced. On single chip level, the wirebonding was carried out, using an automated gold ball-wedge wirebonder (3088iP, ESEC / BESL, Switzerland) to form the vertical electrodes. By modifying the loop shaping parameters, the wire loop can be configured to form either symmetrical 'hair-pin' shaped loops or to get an inclined loop as depicted in Figure 1B. A final multipurpose SU-8 epoxy resin layer was applied and patterned using standard photolithography techniques. The SU-8 acts as a passivation layer to cover the platinum tracks and the bond pads, and is used to form

the micro-well. Only a short section of the wire loop that protrudes sideways into the culturing well is exposed and forms the vertical electrode. As the wire loop shape can be controlled with the bonding parameters, precise control over the vertical position of the electrodes is possible.

Figure 1: A: SEM picture showing the planar and wire electrodes in a circular arrangement around the central measurement site; B: close-up of a planar and wire electrode showing the tilted wire arrangement.

Figure 2: A: Overview of the functional device; B: close-up of the SU-8 well and the electrode configuration

EXPERIMENTAL RESULTS

Figures 1 and 2 show scanning electron micrographs of the wire electrodes and photographs of the fabricated chip (32 mm x 32 mm). The close-up view shows the arrangement of the eight wire electrodes and the eight planar electrodes around the culturing well of 1 mm in diameter that has been defined by a 550- μ m-thick SU-8 layer. Figure 3B schematically shows, how the wire loops protrude into the well at a height of 300 μ m. The loops are inclined by up to 300 μ m. Shapes and heights in the range of 250-600 μ m could be reproducibly and reliably varied by using an automated

wire-bonder.

Figure 3: A: Arrangement of the electrode device on a glass substrate in a culture well made of an epoxy-sealed glass ring; B: schematic overview of the measurement well showing the planar and wire electrodes and the corresponding electrical field lines.

As schematically illustrated in Figure 3, wire electrodes feature larger sensitivity for determining size (and growth) of spheroids as compared to planar electrodes as a consequence of the better positioning of the spheroid in the electric field (see Figures 3 and 4).

We further tested the chip in a “tomographic” mode with two differently sized spheroids at different positions in the well (Figure 5). The inter-electrode impedance was measured between all possible 56 combinations of planar and wire electrodes. The impedance changes with respect to the impedance values of an empty well without a tissue – revealed significant changes at the electrode, which was closest to the tissue. A localization of the tissue can then be used for targeting automated microscopy or for tomographic reconstruction of the tissue shape inside the well.

Figure 4: Comparison of wire and planar electrode configurations. The differential signal was obtained by subtracting the signal magnitudes of measurements without tissues from those with tissues. The wire electrodes provide larger differential signal sensitivity than the planar electrodes.

Figure 5: Normalized impedance changes (normalization has been done by division through values of an empty well without tissue), overlaid to the corresponding micrograph. The location of the tissue can be clearly identified according to the largest impedance changes (thick red lines). Small changes in impedance are depicted as thin blue lines.

CONCLUSION

In this paper we presented a versatile method to fabricate devices with out-of-plane electrodes. By combining a wire-bonding process with standard lithography processes for the metal and SU-8 patterning, devices with arbitrary electrode arrangements can be conveniently fabricated in short time, as compared to using more complex techniques like laser patterning, 3D lithography etc.

A higher sensitivity of the wire electrodes compared to the planar electrodes has been shown and proof-of-principle experiments for “tomographic” applications revealed the position of a microtissue spheroid.

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