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Customised micro-electrode array (MEA) test setup featuring a silicone-casted overlay with two chambers for separated cell seedings

Abstract: Micro-electrode array (MEA) systems are non-invasive platforms for the investigation of electrophysiological properties of cell layers, such as spontaneously active cardiomyocytes. An MEA chip is composed of two-dimensional grids of dot-like electrodes embedded into glass. Here we present a test setup featuring a customised two-chamber silicone overlay. The overlay is designed to be placed on an MEA with two separate electrode fields and enables the seeding of two distinct cell sub-types on the MEA for synchronised drug testing applications while giving the possibility of analysing intersubtype-specific cellular interactions. The overlay has a full size of 10 x 10 x 5 mm (width x length x height), each chamber has a size of 2.5 x 6 x 5 mm ($V = 75 \text{ mm}^3$). The chambers are separated by a wall with a thickness of 0.3 mm. The overlay was manufactured via silicone-casting, utilising a 3D printed model. The model is 3D printed via high accurate digital light processing (DLP). In addition, a DLP 3D printed cover optimises the attachment of the overlay on an MEA. A proof-of-principle of the utilisation of the overlay is demonstrated.

Keywords: Micro-electrode array (MEA), digital light processing (DLP), cardiomyocytes, electrophysiology, drug testing, proarrhythmics risk assessment, silicone casting

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1 Introduction

A micro-electrode array (MEA) system is made of grids of dot-like electrodes and provides a non-invasive, user-friendly platform for the investigation of electrophysiological properties of layers of spontaneously active cells, such as cardiomyocytes. MEA systems enable detailed analysis of beating frequency and conduction velocity of analysed cells through the measurement of the extracellular field potential and can therefore be used for high-throughput measurements of cardiomyocytes, including drug testing, to identify potential targets and the assessment of proarrhythmic risks and side effects [1]. Consequently, MEA technology holds promising opportunities to break new ground for novel therapies in cardiovascular diseases while reducing the need for animal testing [1].

In this study, we use an MEA chip (Figure 1 A) that is composed of two two-dimensional grids of dot-like electrodes (Figure 1 B) embedded into glass. It is of interest to realise the seeding of two separated cell subtypes, one on each grid, for synchronised drug testing applications while allowing the possibility of analysing intersubtype-specific cellular interactions. For that purpose, we present a custom-made silicone overlay manufactured through silicone-casting utilising digital light processing (DLP) 3D printed models. DLP enables high-resolution 3D printing [2] and rapid tooling [3].

2 Materials and Methods

2.1 Design of custom-made overlay for MEA system and scheme of manufacturing process

For an MEA chip (60HDMEA30/10iR-ITO, Multi-Channel Systems, Reutlingen, Germany with two two-dimensional electrode grids (each 5x6 electrodes; 170x210 μm , inter-field

distance: 500 μm) a custom-made digital model of the overlay was designed using computer-aided design (CAD), see Figure 1 C. The overlay has a total size of 10 x 10 x 5 mm (width x length x height) and features two chambers, each of which has a size of 2.5 x 6 x 5 mm ($V = 75 \text{ mm}^3$), that are separated by a wall with a thickness of 0.3 mm. Accessorily, a cover for adequate attachment (fixing of position, sufficient contact) of the silicone overlay on the MEA chip was designed (Figure 1 D). The MEA chip has a glass ring (od = 24 mm) around the electrode grids (Figure 1 A). The cover must friction fit against the glass ring to lightly press the overlay onto the glass surface to provide sufficient contact to seal the chambers of the overlay. Second, the cover features a rectangular window for adequate fluid handling/cell-seeding into the chambers and good oxygen circulation inside.

On the material side, there are two basic requirements that have to be considered when manufacturing the overlay. First, the overlay has to be manufactured from a smooth and flexible material for adequate sealing of the MEA electrode grids. Second, the overlay has to be manufactured from a biocompatible, non-toxic material suitable for long-term (~30 days) cell contact. Silicone is a kind of polymer that meets these requirements. Silicone-casting is an established way of manufacturing silicone components. Because of the minimal thickness of the chamber-separating wall of the overlay of 0.3 mm, there is a need for a flexible cast mold to enable a gentle unpacking of the silicone-casted overlay of the mold after the casting process. For the cover there are other requirements since there will not be direct contact between cells and cover. It should be stiff for frictional connection with the glass ring of the MEA chip and translucent for sufficient light transmission when light microscopy is used as an investigation method. Subsequently, we use a manufacturing process for the overlay described as follows:

1. DLP 3D printing of the overlay model and the cover
2. manufacturing of silicone-cast mold utilising 3D printed overlay model
3. silicone-casting of silicone overlay using a silicone mold

2.2 DLP 3D printing of overlay model and cover for the overlay

A model of the overlay was 3D printed via digital light processing (DLP) utilising a VIDA device (EnvisionTEC GmbH, Gladbeck Germany) and the photopolymer E-Model light (EnvisionTEC GmbH, Gladbeck, Germany) as adequate material for stiff and high-detailed modelling needs. The 3D printed model of the overlay is needed for the manufacturing of a flexible silicone mold, which is finally used for molding

Figure 1: (A) MEA chip with glass ring, (B) two electrode grids of the MEA chip, (C) digital model of overlay and (D) cover

the silicone overlay. In addition, the cover for the overlay is also printed via DLP 3D printing, utilising the biocompatible (class II a, ISO 10993) photopolymer E-Shell 600, which is commonly used for rigid medical products such as hearing aid shells and otoplastics.

For finishing the manufacturing process of the 3D printed model of the overlay and the cover, there is a post-curing process using a UV light curing box (EnvisionTEC GmbH, Gladbeck, Germany) for a time of $t = 10 \text{ min}$ (E-Model light) and $t = 20 \text{ min}$ (E-Shell 600). Measurements of the thickness of the chamber-separating wall of the overlay model is performed using a light microscope (BX51, Olympus Deutschland GmbH, Hamburg, Germany).

2.3 Manufacturing of silicone cast mold and casting of silicone overlay

Silicone cast mold for silicone-casting of biocompatible, soft silicone overlay is manufactured from silicone rubber ADDV M 4601 (R&G Faserverbundwerkstoffe GmbH, Waldenbuch, Germany) as an addition-curing, two-component, room-temperature-vulcanising silicone rubber with high extensibility/stretchability. The DLP 3D printed overlay model is used to form the silicone cast mold. Component mixing (mixing ratio of 9:1 for component A (M 4601-A) and component B (M 4601-B)) and curing of ADDV M 4601 (24 h at ambient atmosphere, $T \sim 21^\circ\text{C}$) is performed in accordance with the manufacturer's instructions.

The silicone overlay is manufactured from two-component soft shore TFC silicon rubber type 26 (Troll Factory, Riede, Germany) through silicone-casting utilising an elastic silicone cast mold. The silicone rubber mixture was manually filled into the silicone mold using a syringe and

needle. Component mixing (mixing ratio 1:1) and curing (48 h at ambient atmosphere, $\sim 21^{\circ}\text{C}$) of TFC silicon rubber were undertaken according to manufacturer instructions. Measurements of the thickness of the chamber-separating wall of the final silicone overlay were performed using light microscope BX51.

The entire test setup of overlay and cover attached to the MEA system is demonstrated. A function test is carried out with the chamber by filling a single chamber of the overlay with (red) cell culture medium and determine if there is adequate sealing.

3 Results

3.1 DLP 3D printing of overlay model and cover for overlay

The overlay model for the following silicon molding was DLP 3D printed and shows high resolution and accuracy (Figure 2 B). The thickness of the chamber-separating wall (Figure 2 C) varies from $\sim 320\ \mu\text{m}$ at the side to $\sim 280\ \mu\text{m}$ in the center of the wall but allows for precise fitting electrode grids. Moreover, the cover for overlay was also DLP 3D printed (Figure 2 A).

3.2 Manufacturing of silicone mold and casting of silicone overlay

The silicone mold was manufactured utilising DLP 3D printed models made of E-Model light. In addition there were no significant problems with manually demolding. The silicone molds were successfully used for silicone-casting of the silicone overlay (Figure 3 A). Due to the highly stretchable silicone material used for the mold, there were no significant problems with manually demolding. However, this manufacturing step was a relative time-consuming process because of the need to ensure the integrity of the thin chamber-separating wall of the overlay. Despite the manufacturing steps being done manually, adequate silicone overlays were successfully produced (Figure 3 B). The thickness of the chamber-separating wall (Figure 3 C) varies from $\sim 280\ \mu\text{m}$ at the side to $\sim 250\ \mu\text{m}$ in the centre of the wall. The difference between the wall thickness of the final silicone overlay and the DLP 3D printed overlay model results from shrinkage of the silicone overlay during the polymerisation/curing process.

Figure 4 shows the completed assembly of the MEA test setup. The silicone-casted overlay was positioned precisely on

the MEA system using a light microscope and the chamber-separating wall is located exactly between the two electrode grids (Figure 4 A). After positioning the overlay, the 3D printed cover is used to fix the overlay's position and provide adequate compression of the overlay, which ensures sufficient contact of the overlay with the glass surface of the MEA. As a result, there is a adequate sealing of the chambers. To carry out a function test, one chamber of the overlay is filled with cell culture medium for 24 h at $T = 37^{\circ}\text{C}$. There is a sufficient sealing, as there is no leakage of fluid into the unfilled chamber (Figure 4 B).

Figure 2: (A) DLP 3D printed cover for overlay of biocompatible (class II a, ISO 10993) photopolymer E-Shell 600; (B) DLP 3D printed model of the overlay of E-Model light for silicone casting; (C) microscope photo of the chamber-separating wall of the overlay model, wall thickness varies from $\sim 320\ \mu\text{m}$ at the side (1) to $\sim 280\ \mu\text{m}$ in the center of the wall (2)

Figure 3: (A) Silicone-casting of the final overlay, (B) finished ready-to-use silicone overlay, (C) chamber-separating wall of the finished silicone overlay with ~280 µm at (1) and ~250 µm at (2)

4 Conclusion

An MEA test setup utilising a custom-made, silicone-casted silicone overlay (10 x 10 x 5 mm) with two chambers ($V = 75 \text{ mm}^3$) for separated cell-seedings on different electrode grids of an MEA chip was successfully developed utilizing a simple, manual silicone casting process. A function test demonstrated a sufficient sealing of the chambers.

Further work will focus on drug testing in cardiac cells utilising the test setup. Different cardiomyocyte subtypes are to be investigated simultaneously.

Figure 4: (A) wall of the overlay separates the two electrode grids of the MEA chip; (B) function test shows adequate sealing of chamber: silicone overlay (light purple) attached to MEA chip and fixed in position via cover, cover overlaps the overlay to ensure an adequate contact with glass surface for sealing of the chambers, one chamber is filled with cell culture medium (red)

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