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ScienceDirect
Biomolecular Engineering 24 (2007) 537–542
Biomolecular Engineering
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Inverse inkjet printed gold micro electrodes for the structured deposition of epithelial cells and fibrin

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Abstract

The micro structured deposition of vital cells is an important challenge in tissue engineering, biosensor technology, and in all research dealing with cell–cell and cell–substrate contacts. Hence, an inkjet printing technology has been developed to manufacture Au-based micro electrodes by sputter coating inversely printed polyester-foils. These electrodes feature minimal structure sizes of 35 μm and consist of an anode and a cathode part. They were used with fibrinogenic epithelial cell suspensions to deposit human keratinocytes (HaCaT), mouse fibroblasts (L-929) and the protein fibrin by applying DC voltage. Subsequently cells were electrophoretically attracted to the anode, following exactly its shape, while the insoluble fibrin was simultaneously precipitated due to the electrically mediated polymerization of the soluble fibrinogen molecule. Furthermore, it was demonstrated that this technique is suitable to co-deposit both cell types in a layered fashion.

The lower voltage boundary for successful deposition was set at approximately 0.8 V needed for the conversion of fibrinogen into fibrin, while the upper voltage boundary was set at approximately 1.85 V, when commencing electrolysis inhibited the deposition of vital cells.

Subsequent to the anodic cell-fibrin deposition, cells were cultivated for up to 4 days and then characterized by FDA + EB staining, methyl violet staining, MNF staining and SEM. The conversion from fibrinogen into fibrin was studied using ATR/FTIR.
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Keywords: Micro electrodes; Inverse inkjet printing; Fibroblasts; HaCaT; Fibrinogen; Fibrin

1. Introduction

During the secondary haemostasis blood clotting occurs due to the thrombin mediated formation of a fibrin network from the blood constituent fibrinogen. This coagulation cascade is a complex biological multi-step process involving a series of enzymes which finally results in the cleavage of two pairs of fibrinopeptides in the central domain of the fibrinogen molecule, which then becomes electrically charged and polymerizes to form a fibrin network entrapping blood cells. This cleaving-process is highly specific which has been demonstrated in crystal structure studies on fibrinogen and fibrin by Russel et al. (2000).

In addition to this enzymatically activated process, it is known that certain materials in contact with blood, like vascular stents can induce blood clotting and finally result in thrombolysis. This process has been first demonstrated by Sawyer et al. in the electrolysis induced clotting of blood (Sawyer et al., 1965). In particular, blood clotting only occurred at the interface of the anode, which has been interpreted as an electron transfer mechanism (Baurschmidt and Schaldach, 1977) from the anode to the fibrinogen molecule, which is unspecificaly cleaved (Rzany and Schaldach, 1999), becomes charged and subsequently polymerizes as found in the natural system.

Recently Haruyama et al. developed a similar methodology to immobilize proteins at electrode surfaces by introducing a peptidic tag into a protein which is then electrochemically reduced (Haruyama et al., 2005).

As has been stated previously, blood cells are entrapped in the formed fibrin network and finally the entire clotting process results in a multitude of intrinsic repair mechanisms to rebuild the damaged tissue. Therefore, adapting this natural cell entrapment process into a tissue engineering concept using electrochemical peptide cleavage might help in the regeneration of certain tissues.

Immobilization of cells on electrode surfaces therefore is of high interest in research dealing with cochlear implants, cardiac pacemakers, nerve-stimulation devices and retinal prosthesis (Rutten et al., 2001; Palanker et al., 2004; Wilkinson, 2004).

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1389-0344/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.bioeng.2007.08.012

In nerve repair fibrin has been described as a potential therapeutic target in certain diseases, such as multiple sclerosis (Rummler and Gupta, 2004; Adams et al., 2004) and might be potentially useful in the early stages of nerve regeneration (Akassoglou and Strickland, 2002).

Therefore, we suggest a methodology for the simultaneous transformation of the soluble fibrinogen from an aqueous solution into the insoluble fibrin attached to an electrode surface and the immobilization of cells on top of the formed fibrin layer. Epithelial cells were used as proof of principle and to demonstrate an elegant way to establish an organotypic co-cultivation system with the additional means to micro structure the formed tissue.

2. Experimental

2.1. Electrode fabrication

Micro structured electrodes were made from non-coated polyester foils used for laser-printing (type "X-70", Folex Imaging). Electrode micro structures were realized by inverse inkjet printing and gold-sputtering. The inverse electrode structure was established by software and printed as black and white on a polyester-foil (HP Deskjet 3520-inkjet printer with HP-print cartridge #27) in 600 dpi resolution. The ink is composed of water (<80.0 wt%), 2-pyrrolidone (<15.0 wt%), isopropanol (<2.5 wt%) and carbon black (<5.0 wt%) (Hewlett-Packard Company, 2003). Printing was followed by gold-sputter coating (Emitech K550) in argon-plasma for several minutes with a sputter-current of 25–35 mA. The gold-coated polyester-foil with the inverse-printed micro-structure was then ultrasonically cleaned in 10% isopropanol to remove the printing ink with the on top gold-layer resulting in the positive-electrode structure. The inverse inkjet printing technique is displayed in Fig. 1. A sample electrode is shown in Fig. 2 which was bent to fit into a 12-well-plate. The electrical conductivity was confirmed after bending, discarding electrodes with no conductivity. Finally, electrodes were stored in 70% ethanol until further use.

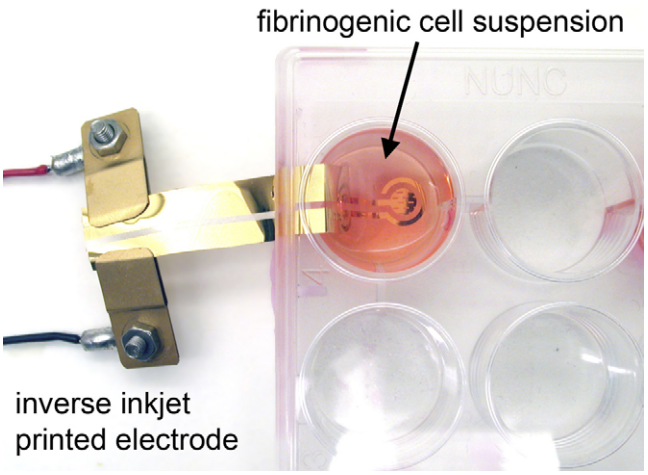

Fig. 2. Sample electrode situated in a 12-well-plate with fibrinogenic cell suspension contacted via gold-coated clamps.

With the inverse inkjet printing technique, electrode structures with a resolution of approximately 35 μm (±6 μm) have been realized.

2.2. Preparation of fibrinogen solution

A 1.0% solution of bovine fibrinogen (fraction I, Sigma–Aldrich) in phosphate buffered saline (PBS, Biochrom) was prepared by dissolving fibrinogen in PBS for several hours under constant stirring at room temperature. This was followed by centrifugation at approximately 750 × *g* to separate remaining undissolved fibrinogen from the solution. The supernatant was pipetted carefully into an empty vial for further use under sterile conditions.

2.3. Characterization of fibrin deposition

The deposition kinetics of the formed fibrin layer on the electrode surface has been estimated visually with a Leica DM 4000M microscope and Leica PL

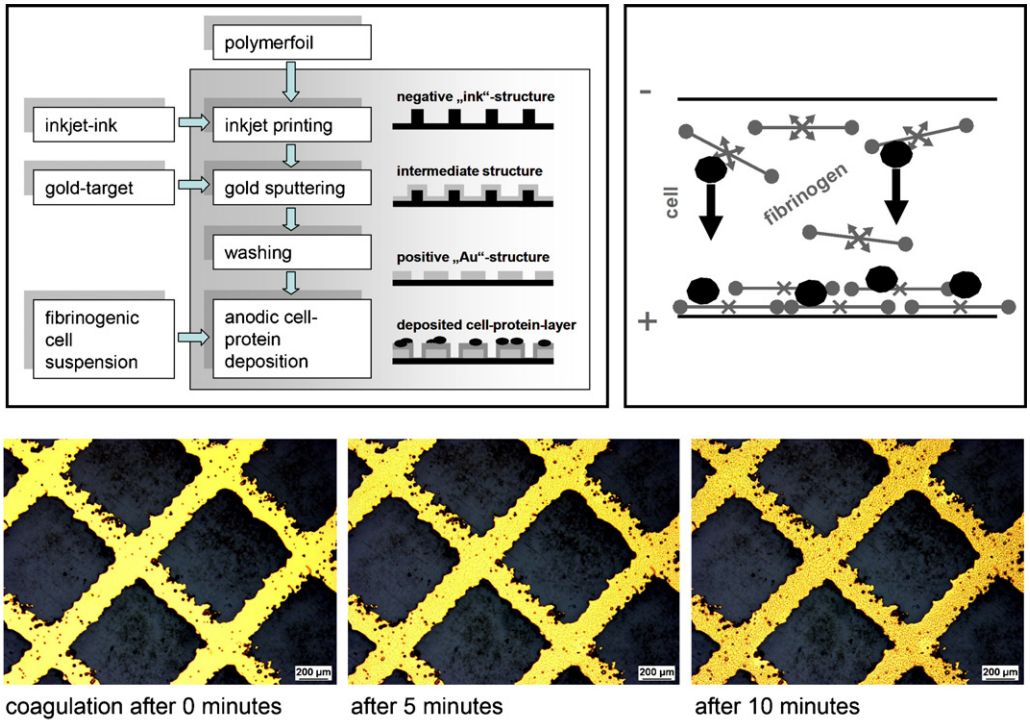

Fig. 1. Processing scheme of the inverse inkjet printing (top) and visual identification of a deposited fibrin-like precipitate on a grid shaped anode (bottom).

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