A multifunctional versatile 3D melanoma model for rapid micro-needle based in situ detection of disease specific biomarkers*

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Introduction

Melanoma is the most lethal skin cancer, having a rapid increase over the past 30 years [1]. The 5-year survival rate after early diagnosis is 99%, while the prognosis for patients with distant metastasis is particularly poor and the survival rate decreases to a dismal 20% [2]. To date, the most effective treatment for melanoma is the early diagnosis, which is followed by surgical resection [3]. Therefore, in order to improve these disappointing statistical figures, it is essential to develop efficient diagnostic tools for rapid detection of disease biomarkers. Minimally invasive microneedles are promising candidates, as they enable rapid and pain-free biomarker detection in situ. However, validating the developed microneedle systems is still challenging. To date, the most commonly used systems for microneedle validation are animal skin models, commercially available skin models and cell culture supernatants (from 2D cultures) [4, 5]. Animals and commercially available skin models can be informative, however their properties such as stiffness, elasticity, porosity, which vary between different patients, for different skin types/complexities and for different ages, cannot be easily tailored [6, 7]. Furthermore, there are limitations when evaluating skin microneedles in supernatants from 2D cell cultures due to the low concentration of the biomarkers which are diluted in the cell culture medium. Additionally, spatial detection is impossible [8]. Consequently, it is of great importance to develop physiologically relevant and versatile models for microneedle

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validation in vitro, that can recapitulate and tune important features of the skin tissue such as stiffness, structure, porosity and pore interconnectivity, elasticity and extracellular matrix composition. Biomaterial based scaffolds are a promising approach to robustly control and tune the above skin tissue futures, and therefore, have great potential as screening tools for novel biomarker detection techniques [9, 10, 11].

The aim of this work was to validate the S100 detection, a marker that is upregulated in melanoma, on a microporous polymer based 3D melanoma model using a novel immunodiagnostic microneedle device.

Methods

3D polymer (PU) based microporous scaffolds ($5 \times 5 \times 2.5\,\mathrm{mm}^3$) were developed as previously described [11] and the metastatic melanoma cell line A-375 was injected and cultured in those scaffolds for 5 weeks. Quantitative assessment of cell viability took place with the MTS metabolic assay and evaluation of cell distribution within the PU matrix was conducted with Scanning Electron Microscopy (SEM). Viable (live) cells were visualised in situ with confocal laser scanning microscopy (CLSM) of several sections of each scaffold. Furthermore, the detection of the S100 marker was carried out with PLA microneedles. The PLA microneedle device was produced, surface modified and coated with the S100 antibody as previously described, followed by the detection of the antigen via immunoassay analysis on the microneedle surface [12].

Results

The 3D microporous scaffolds were able to support long term growth of the A-375 cells, with the majority of them being viable until the culture endpoint. Dense melanoma cell masses adhered to the scaffold pores and were distributed throughout the 3D matrix. Additionally spatial S100 detection was achieved via immunodiagnostic microneedle administration on the surface of the 3D scaffolds. The intense signal detection profile revealed that the antigen capture was highly specific, indicating the ability of the 3D polymeric model to validate the microneedle array.

Discussion & conclusions

Our findings indicate that this 3D polymer based microporous system is a promising tool for ex vivo modelling of metastatic melanoma. The scaffold properties such as porosity, stiffness and elasticity can be easily controlled and tuned, making this scaffolding system a promising candidate for modelling different types of skin (e.g. aged skin). Furthermore, to our knowledge, this is the first time that a 3D in vitro melanoma model is used for validation of biomarker detection with microneedles. Our findings suggest that this 3D microporous melanoma scaffold can be used as a low cost tool for validation/screening of novel cancer detection methods and/or kits, replacing and/or reducing animal testing.

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