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Abstract

3D Bioprinted Hydrogel Sensor towards Rapid Salivary Diagnostics Based on pH Colorimetric Detection †

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Abstract: In this paper, a novel solution of 3D bioprinted hydrogel sensor towards rapid diagnostics of saliva based on pH colorimetric detection is proposed. The hydrogel ink of unique composition of sodium alginate and polyvinylpyrrolidone was developed and printed on biocompatible substrates to achieve a repeatable and robust sensor structure. A colorimetric method was applied to visualize the hydrogel color tunability in a pH range of 5.5–7.5. The biosensor calibration curve was determined showing appropriate performance, as well as experiments with artificial saliva samples containing green tea in different proportions were conducted.

Keywords: hydrogel sensor; 3D bioprinting; pH analysis; optical detection; colorimetry

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1. Introduction

The popularity of hydrogel matrices for biomedical applications has grown rapidly in the last few years. Due to high biocompatibility and ease of fabrication, hydrogel materials are widely used in various biomedical applications e.g., in development of biological sensors of temperature, ethanol, gases, glucose or pH [1,2]. In this regard, especially interesting could be the development of a biocompatible hydrogel sensor to simply evaluate the potential health disfunctions, e.g., based on saliva condition. Such point-of-care device could be fabricated as extremely small sensor, based on modern micromachining technologies, and put directly into the mouth for the rapid intra-oral testing (pH, temperature, biomarkers). In this work, the 3D bio-printed hydrogel pH sensor utilizing colorimetry detection method is shown which responses to the aforementioned demands, being a good base for the development of the fully-featured saliva diagnostic tool.

2. Materials and Methods

The hydrogel layers of the sensor were printed in dedicated square cavities of $10 \times 10 \times 1$ mm^3 developed in biocompatible substrates of $76 \times 26 \times 1.1$ mm^3. The substrates were fabricated by multi-jet 3D printing technique (printer model: Projet 3500 HD Max, 3D Systems, Rock Hill, SC, USA) utilizing photocurable materials–VisiJet M3 Crystal (3D Systems, USA) and VisiJet S300 (3D Systems, USA). The postprocessing of the substrates included i.a. heating in an oil bath in 65 °C for 2–3 h, a detergent wash in elevated temperature (65 °C), and isopropyl alcohol (IPA) cleaning.

The hydrogel ink was composed of 5% (*v*/*v*) sodium alginate and 1% (*v*/*v*) polyvinylpyrrolidone (Sigma Aldrich, St. Louis, MO, USA). The mixture was prepared by stirring (400 RPM) at 60 °C for 60 min. The biosensor was printed using a 3D plotter (BioX, Cellink, San Carlos, CA, USA) with following process parameters: p = 20 kPa, v = 4 mm/s and T = 30 °C (Figure 1a). Afterwards, cross-linking agent 4% calcium chloride

(Warchem, Zakręt, Poland) was mixed with a bromothymol blue pH indicator (Warchem, Poland) and pipetted into the hydrogel structure. The ready-to-use hydrogel sensor was calibrated and next tested with solutions of artificial saliva (Artificial Saliva for Medical and Dental Research, Pickering Labs, Mountain View, CA, USA) and its mixtures with green tea. The images of sensor response to various pH solutions were captured using 12 MP CMOS camera and next analysed utilizing developed software for colorimetric detection (LabVIEW). Red (R), green (G) and blue (B) components were extracted from colour images and presented in graphical and tabularized form (Figure 1b).

(a)

(b)

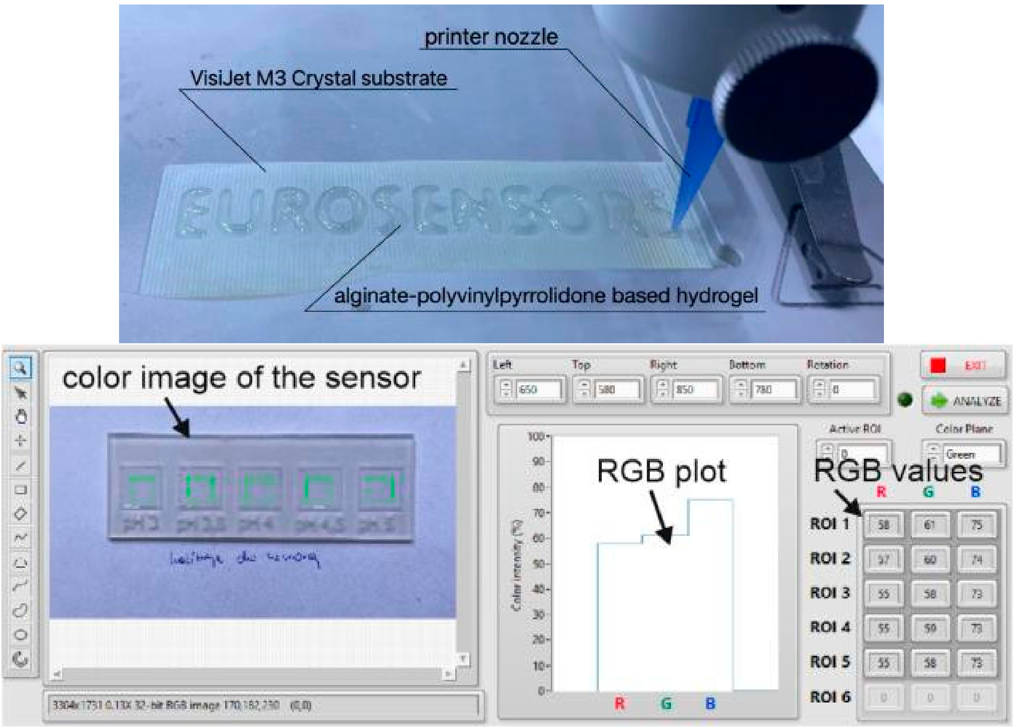
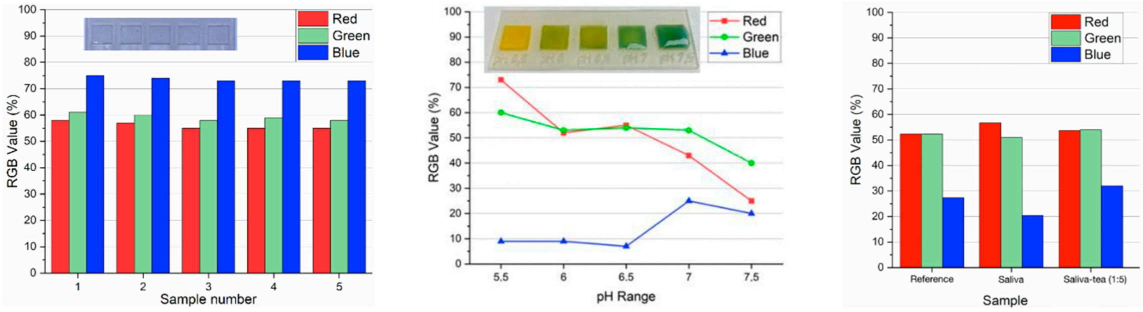


Figure 1. Process of 3D bioprinting-example: (**a**); Front panel of application for colorimetric detection; plate with hydrogel areas selected for analysis—green boxes (left), RGB component analysis for 5 samples—ROI 1 to ROI 5 (right): (**b**).

3. Discussion

At first, the repeatability of sensor fabrication was verified for 5 hydrogel structures and uniform colorimetric response was achieved (Figure 2a). Next, the calibration curve was obtained showing appropriate colour response of the sensor for pH range from 5.5 to 7.5 (Figure 2b). Finally, the samples of artificial saliva mixed with green tea in various v/v proportions were analysed using colorimetric detection (Figure 2c). Intrestingly, the data obtained for saliva and saliva-green tea mixtures indicates a shift towards more alkaline solutions, represented by decrease in R component and increase in B component. The 3D printed hydrogel sensor provides a rapid response (in less than 5 min) and seems to be a promising tool for salivary diagnostics. More detailed results for another saliva-based samples and different pH indicators will be presented in the full-paper.



(**a**) (**b**) (**c**)

Figure 2. Repeatability tests of sensor fabrication–results (5 hydrogel structures shown at the top): (**a**), Calibration curve (real-view of the sensor shown at the top): (**b**), Analysis of artificial saliva samples mixed with green tea in different ratios (reference–no saliva; saliva; saliva with green tea mixed in a ratio 1:5 v/v): (**c**).

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