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# Angiogenesis biomarkers detection on a polymeric 3D printed device

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## Abstract

Polymeric 3D printed chips with intrinsic tuned functionalities were produced and employed for the detection of angiogenesis biomarkers.

## Introduction

The monitoring of tumoral biomarkers involved in pathological angiogenesis is an important task in the establishment of an early diagnostics for patients. Biosensors could represent a promising platform for this purpose. These devices require complex chemical functionalizations to immobilize the biorecognition elements. Here, a novel biosensor consisting in a polymeric 3D-printed chip is presented. The device is printed in a single-step procedure with tuned chemical functionalities, later exploited for the tethering of antibodies. The detection of Vascular Endothelial Growth Factor (VEGF) and Angiopoietin-2 (Ang-2), well-known cancer biomarkers, was performed in order to prove its reliability as a screening tool.

## Materials and Methods

The polymeric chips were realized using a Digital Light Processing 3D printer.[1] The revealment of the biomarkers of interest and the estimation of their Limit of Detection (LOD) and Limit of Quantification (LOQ) were performed by means of a sandwich ELISA-like immunoassay.

## Results

Acrylic acid was added to three resins, to introduce carboxyl groups in the polymeric matrix, to be exploited as anchor point for the immobilization of any bioreceptor that exposes a primary amine.

The printed polymers were tested for their physicochemical properties and protein grafting capabilities, in order to select the top performing formulation that was afterward used for the production of two Lab-on-Chip devices.

The first chip consisted of a microfluidics responsible to convey samples and buffers to an incubation well, where the specific biomolecular interactions took place. The second chip was an alternative version, with three incubation wells simply accessible through an inlet port (see Figure 1).

The LOD evaluated for VEGF and Ang-2 were compared with the cut-off values that are reported in the literature.[2,3] VEGF sensitivity was not high enough to reveal abnormal levels of this growth factor. On the other hand, the LOD relative to Ang-2 (0.8 ng mL$^{-1}$) was below the threshold concentration (2.5 ng mL$^{-1}$), demonstrating a good reliability in a suitable range of values (see Figure 2).

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Key words: Biochemical functionalization; biosensors; 3D-printing; ELISA.

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## Discussion and Conclusions

The combination of the 3D-printing technology with an improvement of photocurable formulations allowed to design a polymeric device having intrinsic tuned

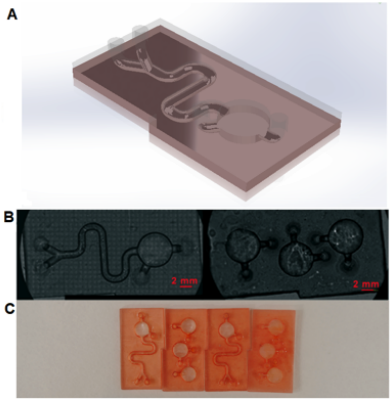


**Figure 1. A) CAD model of the LOC; B) microscope images of the 3D printed microfluidic device; C) images of the printed modular chip.**

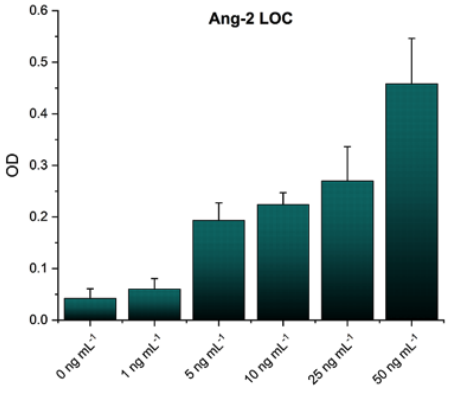


**Figure 2. Detection of Ang-2 through sandwich ELISA-like protocol in the modular LOC: the graph shows the OD signals obtained for different concentrations of Ang-2.**

functionalities with no need for further chemical derivatization. This system was able to specifically recognize Ang-2 with a LOD compatible with the range of concentrations that are required for the identification of a malignant scenario.

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