

## Summary of Dissertation

**Title: Plasma Printing Technology for the Deposition of Functional Coatings for Spatially Controlled Immobilization of Biomolecules**

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The present work describes the investigation and development of a plasma printing technology that allows the deposition of functional plasma-polymerized (pp) thin films aimed at the spatially controlled immobilization of biomolecules. The deposited organic layers exhibited a film thickness from 20 nm to 600 nm, and started at an extension of few millimeters down to a fine linewidth resolution of 50  $\mu\text{m}$  in the case of pp micropatterns, useful in biochip and microfluidics applications. The developed technology, called Surface Atmospheric-Pressure Plasma Printing or SurfAP<sup>3</sup>®, included not only a method, but also the incorporation of the appropriate equipment to synthesize the pp films, in particular, for their deposition as micropatterns. Moreover, the technology offers flexibility, scalability, fast processing and non-toxicity. The fully automated method relies on the combined action of grafting and plasma-induced radical chain-growth polymerization of a thin liquid monomer film placed on the substrate. Some of the limitations of the technology are the minimum linewidth resolution achieved with the current setup and the fact that the pp micropatterns can only be applied on open features and not closed channels, which would limit the biochip and microfluidic applications. In addition, the film thickness for the pp micropatterns is not uniform across the printed pattern, which would also limit the type of biosensing technique that could use these layers.

To achieve a precise amount of liquid and subsequently a desired film thickness, a mist chamber was designed, evaluated and successfully implemented; with the aid of additive manufacturing and plasma-electrolytic polishing. Moreover, the method, along with the monomers selected and operating parameters, allowed tailoring of certain physico-chemical properties of the pp films and micropatterns, in particular thickness, linewidth resolution, chemical groups, and morphology. This investigation is one of the first demonstrations of a controllable process able to tune the cross-linked polymeric chains of plasma-polymers at atmospheric pressure. Nonetheless, it is important to clarify that although the mist chamber production is reproducible, the current fabrication method of selective laser melting (SLM) can be expensive and time consuming. For this reason, it is recommended that if several chambers need to be produced, it would be better to outsource the production and/or change the fabrication and/or the material used (provided that it matches or improves the surface conditions of the metal mist chambers), or consider other mist generation principles.

Plasma polymerized films (as-deposited and after 24 h of immersion in water) from two acrylate-based monomers and their combinations thereof, were characterized via surface analytics. The pp films and micropatterns showed a saturated aliphatic branched structure with incorporated oxygen functionalities, even for the selected monomer that did not contained oxygen in its structure, as well as good stability in aqueous environment. However, the incorporation of nitrogen-containing groups was not detected. The stability was found to be dependent on the oxygen content in the pp films' structure. Different morphological features and increased roughness appeared after immersion, such as ring structures after stored in water, or wrinkles after exposure to buffer solution of pH 7. A thorough search of the relevant literature yielded that



this could be the first report on such nano- and micro-structures induced by immersion in aqueous solutions for these types of films.

To achieve a true micrometer resolution in the case of pp micropatterns via direct writing (maskless deposition), a novel micro atmospheric-pressure plasma source ( $\mu$ APPS) was developed and characterized via preliminary electrical diagnostics. This basic characterization serves as a starting point for further dedicated analysis and development of the device. The source generates a sinusoidal high voltage driven spotlight transient spark (TS) discharge, while the HV electrode is a hollow stainless-steel needle. This provides a narrow footprint (approximate radial extension of 30  $\mu$ m, which in consequence limits the minimum linewidth resolution that can be achieved), while providing low- power and process gas consumption. Thanks to the characteristic high reactivity and efficiency of this kind of microplasma, the  $\mu$ APPS is capable of properly polymerize selected liquid monomers and achieve stable depositions. To the best of the author's knowledge, it is the first time that a sinusoidal-based TS is used for micrometer-range area selective activation and pp-based depositions.

Finally, the pp films and micropatterns were submitted to application tests to prove if the reactive oxygen-rich functional groups present on their surface could be an alternative to the traditional wet chemistry thin film deposition methods for the immobilization of biomolecules. In the case of the larger (millimeter-wide) pp films, SPR and QCM immunosensors for the detection of human serum albumin (HSA) were developed. The pp film-based biosensors prepared showed similar performance as conventional self-assembled monolayers (SAMs) and carboxymethylated dextran (CMD) based sensors, as they proved to have good stability and regenerability. Moreover, the sensors with the plasma copolymer obtain from the monomers tetrahydrofurfuryl methacrylate (THFMA) and 1,2,4-trivinylcyclohexane (TVC) in volume a ratio of THFMA:TVC=0.5, denominated TT12 for simplification, offered the best performance. This was attributed to the larger surface area as product of its characteristic ring-like morphology. With these results, it was demonstrated that not only the amount of functional groups in the pp films is important for successful immobilization of the biomolecules, but also their morphology. In the case of the plasma printed micropatterns (ppTT12), they were subjected to an immobilization proof-of-concept by binding the fluorescent dye Acriflavine. Optical fluorescence microscopy proved interaction between the activated carboxyl groups on the micropattern's surface and primary amines of the dye, evidencing the potential for binding of various proteins. In this way, the prospective application of the SurfAP<sup>3</sup>® technology for the patterning and local functionalization of surfaces aimed at biosensors and microfluidic biochips was demonstrated.