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Comparative study of biocompatibility after surface treatment with kINPen® IND plasma on Acrylonitrile Butadiene Styrene-ABS samples

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Abstract. Surface treatments are widely used techniques for improving the biocompatibility of polymeric materials. Among these treatments, the kINPen® IND cold plasma jet has stood out as an efficient and promising option. The Acrylonitrile Butadiene Styrene (ABS) polymer has favourable mechanical and chemical properties for application in the medical field. However, its low biocompatibility limits its use in certain applications. Therefore, this study presents the application of this treatment to improve its biocompatibility and the cell adhesion assessments. ABS samples were manufactured from 3D printing via FDM, using the GTMax3D Core H4 printer, and subjected to treatment with kINPen® IND. Analysis of wettability using the Ramé-Hart 110-00 goniometer showed an increase in the material's hydrophilicity. The cell adhesion tests with MEF L929 cells showed no significant change in the number of adhered cells in despite of hydrophilicity. Anyway, the results of cold plasma treatment showed that it has a great potential for application in biomaterials for various areas, such as the medical industry and the production of biomedical devices. More cell studies are needed to assess the effectiveness and safety of this method on different materials and applications.

Keywords. *Polymers, ABS, kINPen, Biocompatibility.*

Introduction.

Polymers are macromolecules made up of repeated structural units called monomers, widely used in the automotive, textile, cosmetics, and medical industries. Their classification can be subdivided in various ways, physical properties, chemical structures, or origin for example (1).

In the physical aspect, we have the classifications of thermoplastics, for example, those that soften when heated and then harden when cooled and behave in the same way if subjected to this process again, so they have the characteristic of being recyclable. And thermosets, which also soften and flow, acquiring the shape of the mould, but when subjected to this cycle again do not return to their original characteristics, are insoluble, infusible, and non-recyclable materials (2).

In terms of chemical structure, they are classified as homopolymers when they are made up of just one type of monomer (the basic units that make up polymers) and copolymers when there are two or more monomers (3). In terms of origin, natural polymers come from renewable sources, such as cellulose and proteins, and synthetic polymers are produced through chemical synthesis, such as PVC (polyvinyl chloride), PET (polyethylene terephthalate) and Acrylonitrile Butadiene Styrene (ABS) (4).

Synthetic polymers have gained prominence in the medical field because they can be designed and modified according to the specific needs of each application. Because of this characteristic, the use of these materials is growing every year, for example in medical implants, drug delivery systems and diagnostic devices (5). Among the range of synthetic polymers on the market is ABS, the material used in this paper.

Developed in the mid-1940s by the multinational Dow Chemical Company, ABS is a thermoplastic, non-biodegradable copolymer made up of more than one monomer: acrylonitrile, butadiene, and styrene. It has excellent mechanical characteristics, such as biocompatibility, thermal stability, easy machining, and ductility (6). The use of this polymer has boomed because of advances in 3D printing technologies, especially in the Fused Deposition Modelling (FDM) method, which uses the raw material in the form of spools of filaments and printing is carried out by melting the material in the nozzle of the extruder head, placing the chosen material layer by layer until the desired object is completed, like figure 1 (7-8).

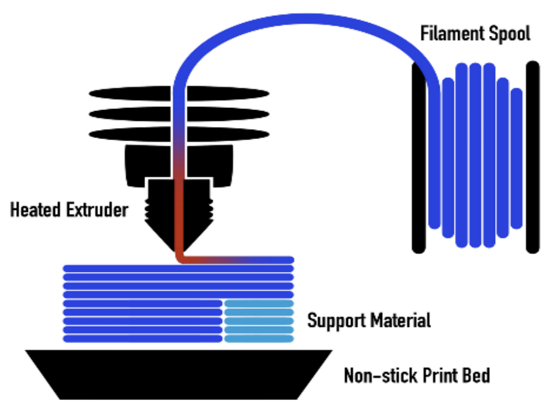

Figure 1: Fused Deposition Modelling (University of Maryland, College Park).

This technique is popularly used in medicine because it has a wide variety of materials, low printing costs and its manufacturing process does not use strong chemical products (9). Its application ranges from aid in surgical preparation to the manufacture of prostheses or tissue engineering applications (10). Because of these characteristics, this was the technique chosen for manufacturing the test specimens.

ABS has a melting temperature below the printing temperature, preventing thermal degradation and loss of bioactivity, a quality that is of the utmost importance when used to manufacture controlled drug release devices (11).

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Although it has biocompatibility as an attribute, ABS is not naturally biocompatible and can cause inflammatory reactions when in direct contact with the human body, an extremely negative effects for use in medical implants unless it is properly subjected to treatments or modifications that increase its biocompatibility, in the case of this study we opted to use the kINPen® IND cold plasma jet treatment (12-13).

kINPen® IND can modify the surface of the polymer, increasing its roughness and creating functional sites that promote cell adhesion and tissue proliferation. It can also improve the material's hydrophilicity, which helps it interact with biological fluids and can improve the number of cells adhered to the polymer's surface (14).

Materials and Methods.

Samples for this study were printed in the shape of a flat disc, measuring 3mm thick and 13mm in diameter using the GTMax3D Core H4 3D printer, where one was subjected to the kINPen® IND surface treatment process and the other was not, for future comparison.

After printing, the specimens were sent to the cleaning process using a Cristófoli ultrasonic cleaner, consisting of three washing cycles lasting 480 seconds each. After a 15-minute drying period, one of the samples was subjected to surface treatment with the kINPen® IND cold plasma jet. It was exposed to the plasma jet for 90 seconds and the kINPen® IND settings were programmed according to the manufacturer: argon gas, 5L/min flow, 50 kHz frequency, 30mA current and maximum voltage of 40kV (14).

Once the plasma was finished, the wettability test was carried out using the Ramé-Hart 110-00 goniometer, the purpose of which is to observe the material's ability to repel or attract water, hydrophobicity and hydrophilicity based on the contact angle, which is the angle between the surface of the material and the liquid interface. This characteristic correlates with the biocompatibility aspect of the material since the more hydrophilic, greater is the ability to attract cells, generating an environment favourable to cell proliferation and cell adhesion, and the more hydrophobic, greater is the repulsion of cells, thus damaging cell adhesion and proliferation (15-16). If the contact angle is between $0^{\circ}<\Theta<90^{\circ}$ the surface is considered hydrophilic and $\Theta=0^{\circ}$ super hydrophilic between $90^{\circ}<\Theta<150^{\circ}$ hydrophobic and between $150^{\circ}<\Theta<180^{\circ}$ super hydrophobic (17).

The second and final test carried out on the samples was cell adhesion - this technique is used to assess a sample's ability to adhere to a cell as a substrate (18). In this test, the samples were submerged in MEF L929 (Mouse Embryonic Fibroblast L929) culture medium and kept in an incubator for 24 hours at 37°C and 5% CO_2. After this test, SEM (Scanning Electron Microscope) was carried out to analyse whether there was cell adhesion and the possible improvement because of treatment with kINPen® IND.

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Results and Discussion.

The results of the wettability test showed that the treated sample had a smaller contact angle compared to the untreated samples, as shown in Table 1, characterising a more hydrophilic surface compared to the untreated sample. This change in the contact angle of the polymer studied is because of the surface treatment in question possibly introduced polar functional groups, such as hydroxyls (-OH), to the surface of the material, thus increasing its affinity with water (19). Figure 2 clearly shows the improvement in the hydrophilicity of ABS, demonstrating that the kINPen® IND plasma jet treatment was effective in accordance with the proposed part of the study.

Table 1:Contact angle with and without kINPen® IND plasma treatment

	Contact angle (ϴ) with surface treatment	**Contact angle (ϴ) without surface treatment**
ABS	31,28°	90,8°

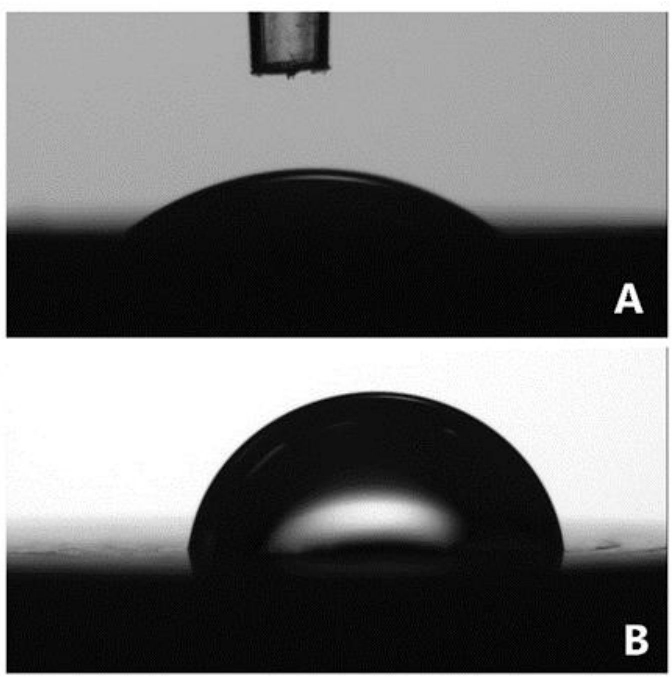

Figure 2: Drop shape of ABS after treatment (A) and before treatment (B)

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The main aim of the cell adhesion test is to assess the ability of cells to adhere to a substrate and consequently improve their survival and proliferation (20). The results of this test, as shown in figure 3, indicate that the treated sample showed cell adhesion, but that the untreated sample did not show such a significant change compared to the results obtained from the wettability test.

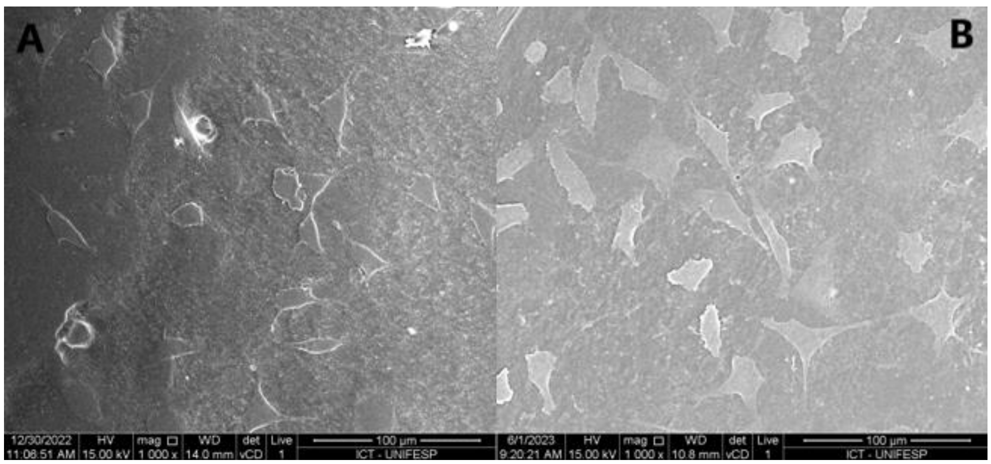

Figure 3: Cell adhesion in ABS after plasma treatment (A) and without treatment (B)

Conclusion.

It can be concluded from the results that surface treatment with kINPen® IND cold plasma jet can be an effective strategy for improving hydrophilicity on the ABS surface. However, in terms of cell adhesion, it was not possible to correlate the same improvement, as it did not show a significant increase in adhered cells as presented in the literature.

However, according to the study presented by Menzies and Jones (2010) two situations were addressed, the first being that the more hydrophilic the material the better it would be for its application, in this case for use in dental implants where there was an improvement in osseointegration and a reduction in inflammation due to the increase in cell adhesion, while the second situation was for use in intraocular lenses where the hydrophilicity of the material reduced cell adhesion, improving optical performance.

From this analysis, the behaviour of the material studied in relation to wettability and cell adhesion will always be relevant, but the justification that one characteristic is better than the other can only be affirmed with the specification of the application, making it clear that each result is unique and what may be bad for a given objective is good for another. The technique used for surface treatment has shown great potential for application in various areas, such as the medical industry and the production of biomedical devices. However, more studies are needed to assess the effectiveness and safety of this method on different materials and applications.

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