THIN FILM DEPOSITION • PLASMA PROCESSING • VACUUM INSTRUMENTATION

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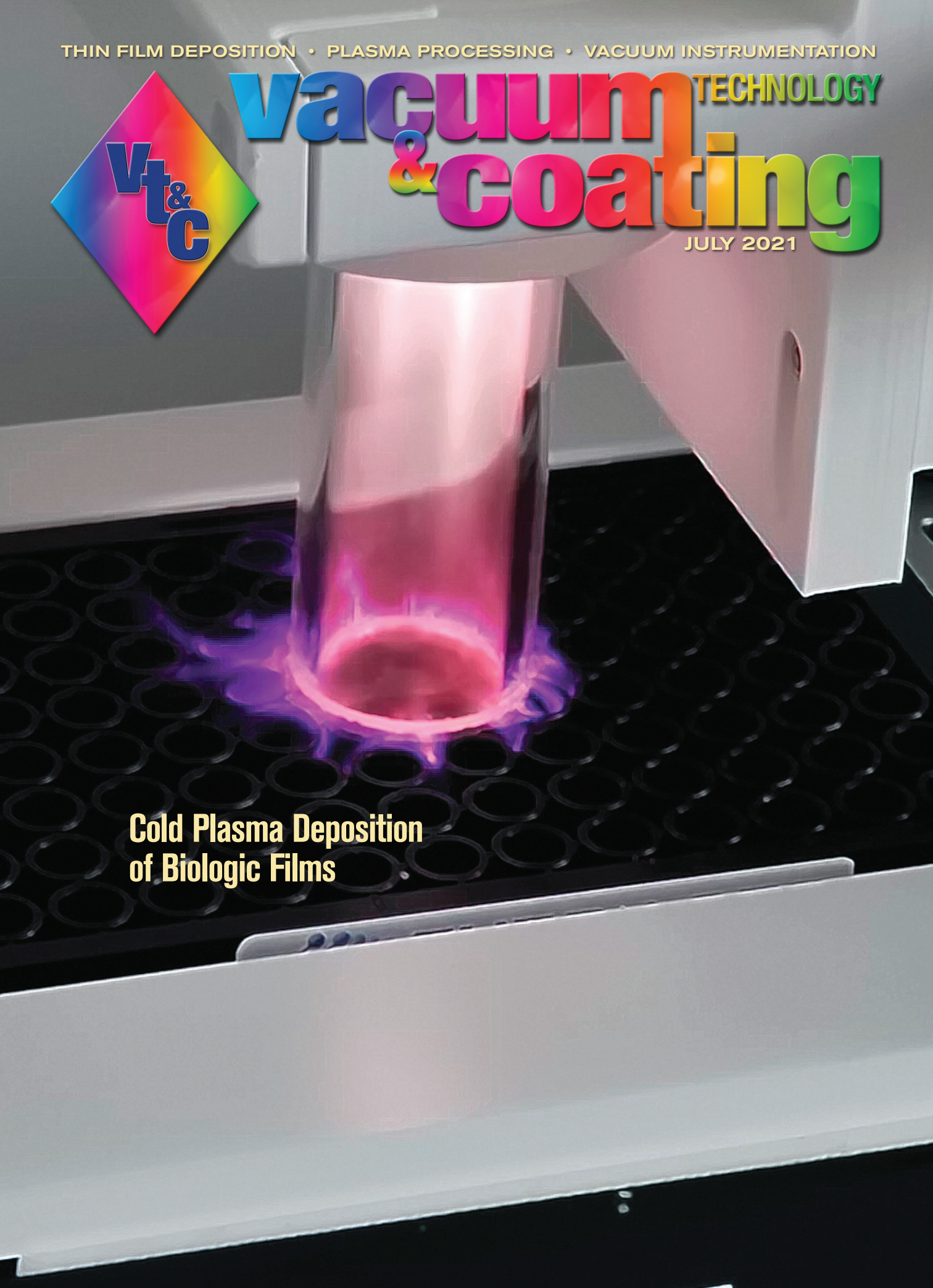
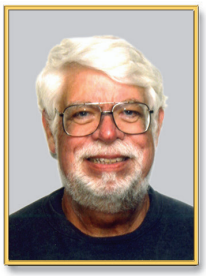
JULY 2021



Cold Plasma Deposition of Biologic Films

Guides to Vacuum Technology



By Steve Hansen, *Contributing Editor*

Cold Plasma Deposition of Biologic Films

Soft plasmas for delicate molecules

The four columns of May through August 2020 dealt with the basics of atmospheric pressure "cold" plasmas, ways to generate this type of plasma and some applications. The generation mechanisms included corona discharges, the dielectric barrier discharge (DBD) and the atmospheric pressure plasma jet (APPJ). Applications included surface modification for static control, improved wettability and adhesion, sterilization, the deposition through plasma enhanced CVD of protective coatings of various types of inorganic coatings such as passivation layers, and so forth.

This column will look at the deposition of biologic films such as collagens, antibiotics and other pharmaceuticals. Such films are important to medical practice.

Deposition of Structurally Well-Defined Polyacrylic Acid Films

The column of May 2021 discussed various methods for metering or measuring the flow of liquid precursors. One example that was briefly touched on was a dielectric barrier discharge device used for the deposition of "structurally well defined" polyacrylic acid films using acrylic acid as a precursor. The device, as described by Ward *et al.* [1], is depicted in F**igure 1**. The liquid precursor was delivered from a syringe pump into an ultrasonic nebulizer where the exit of the nebulizer was centered within and coplanar with the upper, grounded, electrode.

As noted in the June 2020 column, this type of discharge can be either filamentary (think of numerous miniature lightning strikes between the electrodes) or it can be in the form of a uniform glow discharge. The former condition concentrates the reactants into the relatively intense and non-homogeneous filaments. The latter, homogeneous, situation has been described using a variety of terms but the paper uses the term atmospheric pressure glow discharge or APGD,

The described device uses an APGD. This was key to producing the desired "structurally well defined" polymer film. The production of an APGD requires the optimization of a number of factors: voltage, waveform, frequency, electrode finish, electrode separation, dielectric thickness and various factors that are associated with the substrate. It is also only suitable for flat substrates.

The experiment showed that, in addition to being highly wettable, the poly(acrylic acid) plasma polymer possesses good adhesive and gas barrier characteristics.

Herbert *et al.* [2] in their 2011 paper note that prior to the mid 1990s, plasma enhanced CVD processes were limited to the vacuum deposition of relatively simple coatings such as silicon nitride and oxides because of fragmentation of the vaporized precursors. This changed when pulsed PECVD processes were introduced. The pulses permitted active species to be created during the pulse but the pulse did not contain enough energy to fragment all of the bonds in the precursor molecules. They note

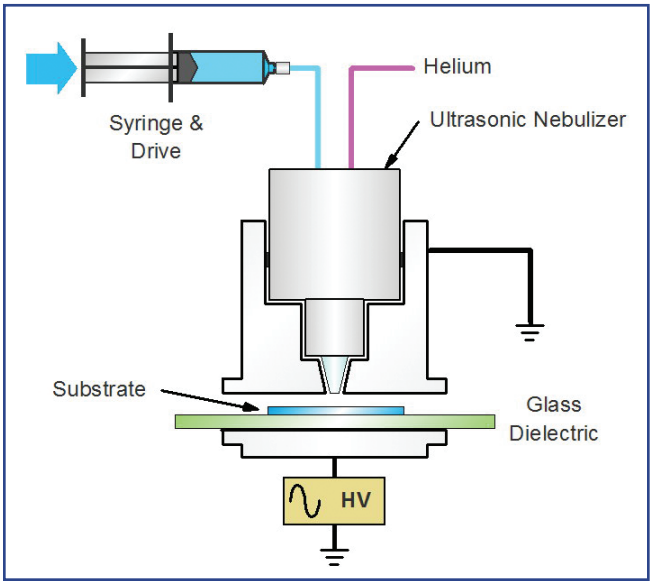


Figure 1. Dielectric barrier glow discharge device for depositing polyacrylic acid films. Illustration based on [1].

that Forch *et al.* [3] described this as a "soft plasma assisted modification" in which the precursor molecules do not fragment, but are activated predominantly at particular reaction sites, which then undergo polymerization reactions during the plasma "off period."

The authors go on to state:

It is therefore possible to differentiate "polymerization sites" in the monomer molecule from "functional sites" containing the key molecular properties to be replicated in the polymer. This led to the identification of "soft plasma conditions" in which it is possible to carry out polymerization with the least damage to the functional chemistry. Thus, control of substrate temperature, reactant pressure and flow rate, absorbed continuous wave power and location of substrates at varying distance from the plasma region have all been used to bring greater levels of control to the polymerization process.

The APGD example above made use of this principle at atmospheric pressure by combining a low energy helium glow discharge plasma with a liquid aerosol delivery system for the precursor.

Some researchers have found that a higher level of flexibility can been obtained by replacing the parallel plate electrodes with a needle or pin electrode scheme.

Pin Electrode Devices

Unlike the previous example of the planar dielectric barrier device, in a unipolar pin electrode arrangement there is only one connected electrode. **Figure 2** shows a very simple configuration devised by Lu *et al.* [3]. The hardware uses a glass syringe body and the intended use is for biomedical applications.

In this arrangement, no physical counter-electrode in the form of a conductive plane needs to be placed to oppose the needle as

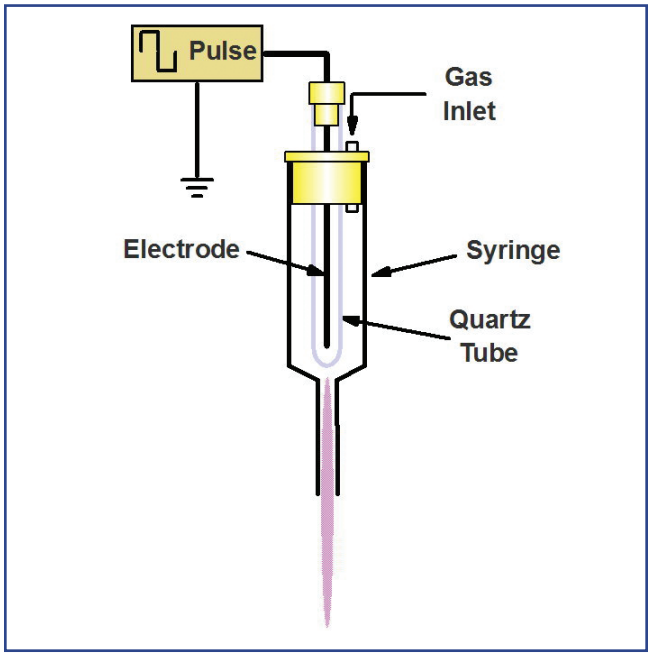


Figure 2. Single electrode APPJ. Illustration based on [3].

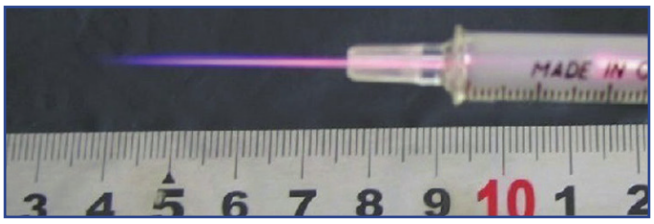


Figure 3. Single electrode APPJ in operation showing the plume. Photograph from [4], open source.

the high electric field at the pin tip sees the surrounding ambient as the "ground plane." The pin will discharge freely from the needle tip into the tube.

Figure 3 shows this type of APPJ in operation. The syringe inner diameter is 6 mm and the quartz tube has an outer diameter of 4 mm. Separation between the quartz tip and the 1.2 mm diameter orifice is 1 cm. Power is pulsed DC with pulse widths as short as 200 ns. Voltage was up to 10 kV and frequency up to 10 kHz. Because the device has only one electrode, the discharge is generated between the HV electrode and the surrounding air.

The J-Plasma Electrosurgical Device

Electrosurgical devices have a fairly long history. I remember getting a wart removed from a finger many years ago where the doctor used such an instrument.

There are two general types of electrosurgical devices. Unipolar devices, as described above, will have a return path through the patient's body and thereby to ground. These instruments are effective where cutting and coagulation are required and stray currents do not pose a risk to the patient. Bipolar devices have the return incorporated in the surgical instrument. The effects are therefore restricted to the small area of tissue between the two electrodes.

An interesting device that uses a unipolar pin electrode and helium as the working gas is the J-Plasma® cold plasma surgical device by Apyx Medical [5] of Clearwater, Florida. Apyx grew from Bovie Medical Corp. There is a related device for cosmetic surgery that is marketed under the Renuvion® name.

In all cases, the frequency of the electrical excitation must be over about 100 kHz in order to eliminate any sensation through the nerves of the patient. Typical frequencies are in the range of 500 kHz.

The J-Plasma tool combines a knife with a unipolar plasma source so that the surgeon may quickly switch between cutting and the functions provided by the plasma. In addition, the plasma can also be activated while the knife is exposed. This last configuration resembles an electrosurgical knife approach. However, with the addition of the inert gas and plasma, cuts made show virtually no eschar (dead skin that sheds from the wound) with very little collateral damage along the side walls of the cut. Furthermore, the cutting speed is considerably faster, with less mechanical cutting resistance as compared to when the knife blade is not electrically energized. Hemostasis (the physiological process that stops bleeding at the site of an injury while maintaining normal blood flow elsewhere in the circulatory system) is also affected during this process.

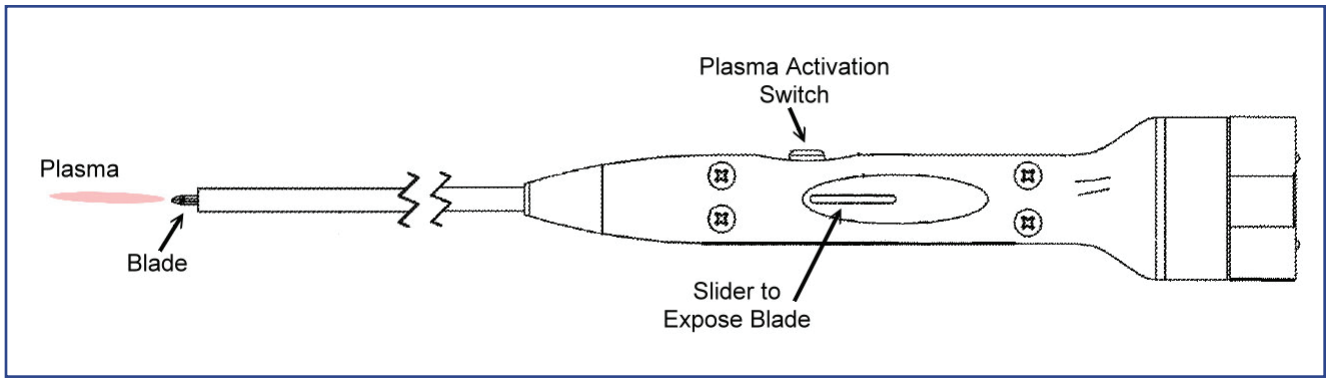


Figure 4. J-Plasma electrosurgical device with unipolar plasma. Illustration adapted from [6].

Figure 4, adapted from [6], depicts the configuration of one version of the J-Plasma. It consists of a handle with extension tube. At the exit of the tube is the retractable blade. The blade serves as the pin electrode and is coupled to a RF generator that is in a stand-alone console. The blade, when extended, serves as a scalpel. In use, when the blade is retracted, the device may be used for controlled ablation and coagulation. When the blade is extended, it may be used for enhanced precision and cutting with the plasma present.

The TheraDep BioDep Process and Device

Getting back to PECVD a young company called TheraDep [7] (Tipperary, Ireland and San Jose, CA) has been active in the development of devices for biofilm deposition using unipolar pin electrodes along with a nebulizer. This basically in an evolution of the device as described in [1] but exchanging the parallel electrode APGD configuration with two pointed electrodes in parallel. **Figure 5** depicts the configuration.

The following is largely drawn from O'Sullivan *et al.* [8]. In the Teflon head there are two pin electrodes and a parallel path nebulizer (Burgener Research, Mississauga, Ontario, Canada). The material to be coated is supplied as a solution to the nebulizer. Helium (preferably, or other inert gas) is directed into the nebulizer and past the two electrodes. Typical values for flow rates are 40 µL/min for the solution, 2 slm of helium to the nebulizer and 8 slm total for the two pin electrodes.

Below this assembly is an acrylic plastic mixing tube with in inside diameter of 19 mm and a length of 35 mm.

Deposition of a Biologic Onto a Surface

Microplates are important tools in biological research and analytical operations. A typical microplate is made from molded plastic such as polystyrene and have an array of shallow wells. They might be thought of like a matrix of small test tubes. The as-molded plastic is not conducive to cell attachment so plasma wettability treatments are often used to promote wetting. This is generally not enough, so additional treatments are required. Typically, this has been done by coating the microplate wells with attachment promoters such as collagen. The collagen surface is more biologically "natural" than plastic.

The usual process for coating involves a fairly complex protocol based on wet processes. After the plasma treatment for wetting enhancement, a dilute solution of collagen is pipetted into each well. It is then allowed to adhere. After about an hour at room temperature, the residual collagen solution is aspirated without disturbing the adherent coating. This is then followed by washing. Further steps may be taken if the plates are to be stored. All of the process steps take place in an aseptic clean room.

The BioDep process converts all of the above to one unified process step. As described in the paper, "it was hypothesized that direct plasma deposition technique would allow a thin layer of collagen to be evenly applied to the surface, with process advantages of speed and adherence." The study bore this out.

Figure 6 shows the head of the BioDep apparatus. **Figure 7** is a photograph of the deposition head as mounted on a computer controlled coating station.

The following contains the significant results from the study.

The electrical parameters were 5.28 kV and a maximum current of 174 mA. The calculated plasma output power was 4.746 watts.

In comparing the wet processed with the plasma processed coatings using FTIR, no shifts in the peaks were noticed, indicating that the there were no measurable chemical changes due

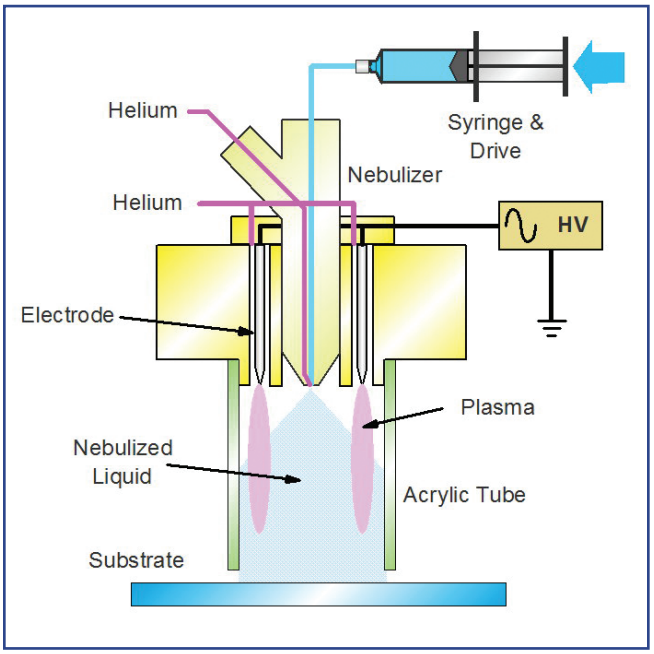


Figure 5. ThereDep BioDep apparatus.

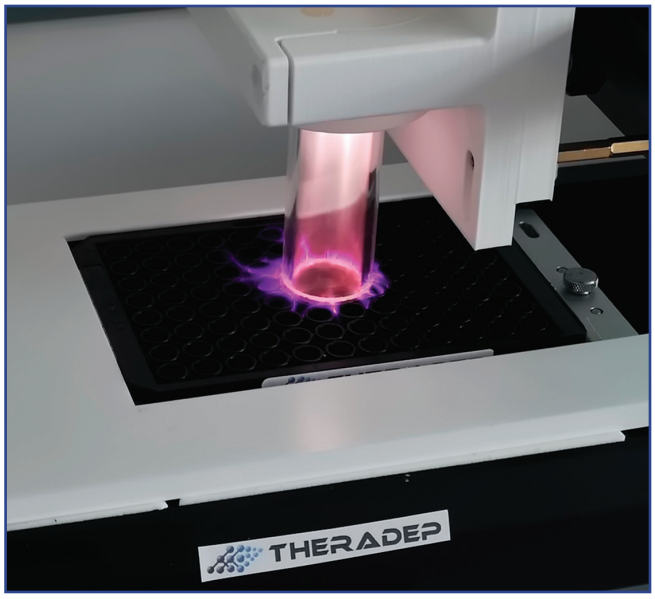


Figure 6. BioDep deposition head poised above a 96 well plate. Image courtesy of TheraDep.

to the plasma processing. Contact angle measurements showed adequate performance for each type of process.

In a functional test, three plates were used. The first was uncoated. The other two were coated with rat tail collagen, one commercially prepared and one plasma processed. This would show if there was a significant difference between the plasma coating and the commercially available collagen-coated plates. **Figure 8** shows optical micrographs of the test plates. On the control plate with no coating, the cells clumped, indicating that the surface was not appropriate for their growth. The commercially coated and plasma coated plates showed comparable (favorable) results.

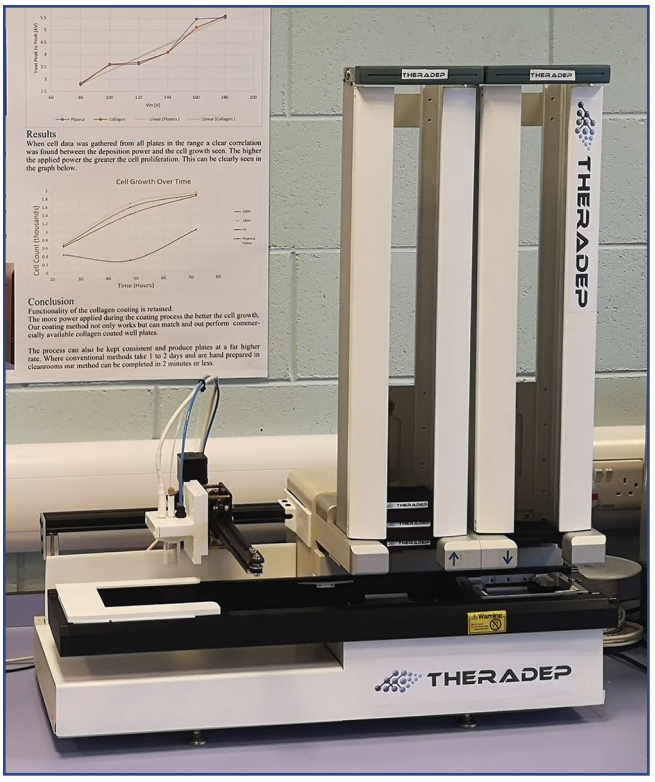


Figure 7. Complete BioDep plate stacking facility for coating microplates. Image courtesy of TheraDep.

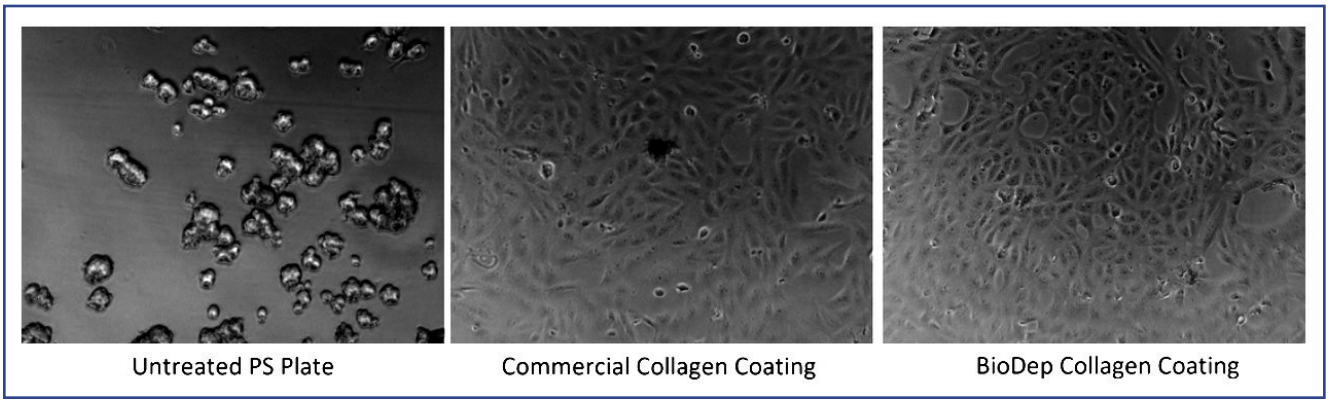


Figure 8. Comparison of uncoated, commercially coated and BioDep coated microplates. Images courtesy of TheraDep.

Overall, the plasma coated plates showed better uniformity than the wet coated films. With comparable performance characteristics, the plasma process saved considerable time (a couple of minutes vs. hours), labor and the need for an aseptic clean room.

Medical Applications

While the BioDep process was designed for the application of biologic films such as collagen to laboratory apparatus, work is also proceeding on the use of this process to directly deposit pharmaceuticals such as antibiotics to a range of objects such as surgical sites or implants.

Some initial work was performed by O'Neill *et al.* [9] on wound healing in rats using plasma deposited collagen. This used the previously mentioned J-Plasma device (see **Figure 3**) with a nebulizer and mixing chamber add-on. This was functionally similar to the later BioDep apparatus.

A dry, adherent coating was produced and analysis showed that the chemical properties of the dissolved collagen were retained. The team found that enhanced healing was shown in 2 out of 3 rats thereby warranting further work.

More recently Los *et al.* [10] discuss the delivery of antibiotics to surfaces and the resulting antimicrobial efficacy of these films.

A patent on the TheraDep device for wound healing by O'Keefe *et al.* [11] combines all of this and discusses several scenarios. The overarching scenario is for wound healing as described above where the plasma is used to sterilize the area, coagulate blood and deposit a bioresorbable coating to seal the wound area. Additionally, the coating can also include other active components to facilitate the healing process. The bioresorbable coatings can include collagen, blood plasma or chitosan. Other materials may be substituted or used in addition. These can include a protein, a biopolymer or others. For active materials, these can include drugs, enzymes and so forth. The result is a single step, multi-function process. Just what the doctor would order.

TheraDep has formed a unit, AerMedX [12] that is tasked to work on these and similar applications. A prototype hand-held device is depicted in **Figure 9**.

Summary

Biologic films may be deposited at atmospheric pressure with plasma enhanced chemical vapor deposition, By nebulizing an aqueous solution and with modest power and unipolar electrodes, these films retain the effectiveness of the of the original biologic material. For application to diagnostic materials such as microplates, the result is a significant improvement in time and complexity as compared with wet methods. Such films can also be applied to patients in surgical applications such as wound healing.

Acknowledgements

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Figure 9. AerMedX hand-held device for wound healing and other applications. Image courtesy of AerMedX.

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