State of the art in nonthermal plasma processing for biomedical applications: Can it help fight viral pandemics like COVID-19?

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Abstract
Plasma processing finds widespread biomedical applications, such as the design of biosensors, antibiofouling surfaces, controlled drug delivery systems, and in plasma sterilizers. In the present coronavirus disease (COVID-19) situation, the prospect of applying plasma processes like surface activation, plasma grafting, plasma-enhanced chemical vapor deposition/plasma polymerization, surface etching, plasma immersion ion implantation, crosslinking, and plasma decontamination to provide timely solutions in the form of better antiviral alternatives, practical diagnostic tools, and reusable personal protective equipment is worth exploring. Herein, the role of nonthermal plasmas and their contributions toward healthcare are timely reviewed to engage different communities in assisting healthcare associates and clinicians, not only to combat the current COVID-19 pandemic but also to prevent similar kinds of future outbreaks.

KEYWORDS
antibiofouling, biomedical applications of plasmas, biosensors, COVID-19, plasma decontamination, plasma-enhanced chemical vapor deposition

1 INTRODUCTION
The healthcare industry has, over the years, transitioned into one of the most significant and dominant sectors, with business expected to grow up to USD 10 trillion by 2022. [1] With the spurt in lifestyle-related diseases, ailments caused by rising pollution levels, and a general human effort toward attaining higher life expectancies, it has become essential to come up with unique treatment strategies and rapid bioanalyte detection techniques that can provide us information about any impending diseases or physiological anomalies with a high degree of precision and accuracy. One of the processes that have come to the forefront owing to its versatility is plasma processing technology, a highly dependable technique for materials processing, including deposition of metallic, polymer, and hybrid inorganic–organic thin films, polymer modifications, surface activation, etching, surface disinfection, and so forth. These applications
have permeated the world of healthcare, making their presence felt in domains as diverse as sterilization of food and medical products, microfluidics, biosensors, antibiofouling surfaces for medical devices (MDs; e.g., implants, catheters, meshes), wastewater treatment, esthetic surgeries, wound healing, and oncotherapy. In fact, the widespread footprint of this technology can be gauged by the fact that the global cold plasma market size currently stands at an impressive USD 1.6 billion and is projected to reach USD 3.4 billion by 2025, at a compound annual growth rate of ~16%. The key drivers for the significant growth of cold plasma technology include economic viability, low footprint, energy efficiency, and eco-friendly (dry process, low consumption of water, and chemicals) and worker-friendly attributes. [2]

The coronavirus disease (COVID-19) outbreak caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus has affected 220 countries, with more than 58 million cases of infections and over 3.3 million deaths reported worldwide till May 09, 2021, and counting. [3,4] New cases are emerging at an alarming rate of 0.9 million and over 15000 deaths per day, adversely impacting the global economy in the process. A combination of broad-spectrum antibiotics and antivirals is currently in vogue as standard care therapy along with ventilators to handle breathing difficulties. But with an increasing number of recurring infections in COVID-19 patients, it has become critical to urgently develop novel, innovative, and efficient antiviral strategies to face this crisis within the stipulated time, as per the mandate of the World Health Organization. On this note, the noninvasive approaches of nonthermal gas plasma-assisted generation of reactive oxygen and reactive nitrogen species (RONS) are highly potent to inactivate a full spectrum of microorganisms, [5,6] including vegetative, [7] different microbial spores, [8] fungi and yeast, [9] biofilm-forming bacteria, [10] and deadly viruses. [11,12] The antimicrobial effects of nonthermal plasmas are rooted in a unique synergy of its transient species including RONS, free radicals, metastables, and photons. [13] Weidinger and Kozlov in their interesting review consolidated old and new ideas on the chemical, physiological, and pathological role of exogenous RONS for a better understanding of their properties and specific activities. The critical review reveals that the reactions of primary RONS (superoxide dismutase and catalase) with biomolecules are reversible, making them ideal for physiological/pathophysiological intracellular signaling. However, the secondary RONS are highly potent, difficult to regulate generation and concentration, and their damaging effects are irreversible. Whether RONS have a signal-transducing or damaging effect is primarily defined by their quality, being primary or secondary RONS, and only secondly by their quantity.

In this review, we focus on some of the important healthcare applications where the nonthermal plasma has played its part, the state of the art and future prospects, as well as its potential role in combating the ongoing global crisis of the COVID-19 pandemic.

2 | PLASMA PROCESSING OF MATERIALS

Plasma, the fourth state of matter, is a quasi-neutral gas medium comprising of energetic electrons, fragmented ions, neutral free radicals, metastables, and photons. There are two main groups of laboratory plasmas: (i) low-temperature plasma, for example, gas discharge plasma, and (ii) high-temperature plasma, for example, fusion plasmas. Gas discharge plasmas can be further categorized into equilibrium (thermal) and nonequilibrium (nonthermal) plasmas. In nonthermal plasma (cold plasma), the heavy species (ions and neutrals) exist in a thermal energy distribution of 300 K, whereas the electron energy distribution is close to that of several thousand K. In fact, the majority of electrical energy goes into the production of energetic electrons, instead of heating the entire gas stream. Elementary processes with high activation energies of several eV are thereby possible at low temperatures using low-pressure nonthermal plasmas. As a result, there is almost no thermal load on thermosensitive polymeric materials during cold plasma treatment. Thus, nonthermal plasmas have been extensively employed in the modification of polymers for various technological and industrial applications including biomedical and healthcare applications. [14-19]

In terms of operating pressure, plasma can be categorized into low-pressure and atmospheric-pressure plasmas. Atmospheric-pressure plasmas, in particular cold atmospheric-pressure plasmas (CAPs), are of considerable interest, as, unlike low-pressure plasmas, they do not require closed reactors or evacuation under high vacuum, and can be operated under atmospheric conditions. Besides being relatively cost-effective, they offer flexibility in terms of substrate size. Techniques such as dielectric barrier discharge (DBD), atmospheric-pressure plasma jet, plasma needle, and plasma pencil have been traditionally used to generate CAPs. The nonthermal CAP operates at an ambient temperature (<40°C) that can be achieved using various electrode configurations discussed elsewhere. [20]

Figure 1 illustrates different configurations of typical laboratory-scale low- and atmospheric-pressure plasma setups for surface activation, surface functionalization,
thin-film coating, and etching. Under low pressure (<10^{-2} \text{ mbar}), the radiofrequency (RF) plasma setup depicted in Figure 1a produces a capacitively coupled plasma using two parallel-plate metal electrodes separated by a discharge gap, whereas an inductively coupled plasma (ICP) uses an external coil wound on a quartz tubular reactor while the substrate is grounded (Figure 1b). Both systems are driven at 13.56 MHz RF. Figure 1c presents a microwave plasma setup, which is driven at 2.45 GHz after pumping down the reactor to <10^{-6} \text{ mbar}. Figure 1d depicts an RF magnetron sputtering system used for deposition of metals, oxides, and polymers from their respective targets. A matching network is required to match an RF/MW generator with its impedance of 50 \Omega to the load (plasma chamber) and adjusted in such a way that the forward power to the plasma is maximized while minimizing the power reflected back to the generator. To avoid using expensive vacuum systems and pressure gauges, atmospheric-pressure dielectric barrier plasma generation using two metallic electrodes covered with a single (Figure 1e) or two dielectric layers (Figure 1f) (to avoid glow to arc transition) can be used for substrate etching, activation, deposition, and decontamination applications. Low-temperature plasma jet using two rings (Figure 1g) and needle-to-ring (Figure 1h) configurations are used for direct and indirect delivery of reactive species to liquid mediums, disinfection, and clinical studies. These different configurations of nonthermal plasma systems have been widely investigated, with or without gas mixtures, for a variety of applications, for example material treatments, disinfection, cancer treatment, and so forth, in laboratory as well as in clinical settings.\[21-24\]

Plasma processing of materials, and in particular polymeric materials, includes six fundamental processes: (i) surface activation/functionalization using chemically reactive gases, (ii) surface functionalization by grafting reaction, (iii) plasma-enhanced chemical vapor deposition (PECVD) or plasma polymerization (PP), (iv) plasma cross-linking of polymers, (v) plasma etching/cleaning, and
(vi) plasma immersion ion implantation of polymers for direct covalent attachment of biomolecules, hydrogels, and inorganic species. Depending on the physicochemical properties of the precursor gas, material, and subsequent processing steps, any of these processes or their combinations can be utilized at different stages of manufacturing or for performance enhancement of devices. As illustrated in Figure 2, combinations of different plasma processes have found use in the engineering field and in enhancing the performance of devices used in healthcare and biomedical applications.

3 | MICROFLUIDIC DEVICES

Microfluidics refers to the design, manufacture, and formulation of devices with dimensions within the millimeters to micrometer range, and processes capable of handling minute volumes of fluid in the microliters to picoliters range. Microfluidics-based miniaturized analysis systems, called micro total analysis systems or “lab on a chip” (LOC), have been of particular interest. “LOC” incorporates a microfluidic system on a microscale chip and includes microchannels, mixers, reservoirs, diffusion chambers, integrated electrodes, pumps, valves, detectors, and so forth. It aids in automating standard laboratory processes and ensures minimum use of precious reagents (e.g., enzymes, biological samples), making the technology fast and economical.

These systems provide a powerful platform for biological assays having numerous applications, including DNA sequencing, polymerase chain reaction (PCR), DNA separation, enzymatic assays, immunoassays, blood cell counting, blood cell sorting, and cell culture. Similarly, microfluidics-based immunosensors, for example, enzyme-linked immunosorbent assay (ELISA), have improved the efficiency in automatic detection of analytes with shorter assay times and increased detection sensitivity. Recently, microfluidic PCR chips have also proved their worth as effective point-of-care diagnostic tools for rapid detection of COVID-19 virus from trace amounts of samples. Expanding on these concepts, companies such as Randox Laboratories have invested in path-breaking technologies, such as the Biochip Array Technology, which is capable of simultaneous multianalyte diagnostic testing within the field of clinical research and testing, drugs of abuse screening, and DNA analysis. After the recent crisis of COVID-19, rapid developments have been made to the technology to come up with a highly innovative viral respiratory tract infection array for screening of COVID-19 (SARS-CoV-2) viral strain. This can help minimize the chances of spread of the disease through early identification of a potentially infected person.

The traditional materials used in microfluidic device fabrication are glass and silicon. But polymer-based devices have garnered sizeable attention due to their low cost, ease of fabrication, good bonding to other surfaces, and disposable applications. The inherent flexibility of polymer substrates facilitates the integration of various functionalities and actuators such as microvalves and micropumps in Biomedical microelectromechanical systems and LOC devices. A range of polymer substrates including polydimethylsiloxane (PDMS), polymethylmethacrylate (PMMA), poly-carbonate (PC), and polyethyleneterephthalate (PET) can be used for the fabrication of microfluidics devices. Nonthermal plasma processing (surface activation, coating, etching, etc.) plays its part as a highly efficient polymer surface treatment technique that can be employed both in the fabrication stage as well as for performance enhancement of devices. Such low cost, disposable polymeric microfluidic devices can prove remarkably useful in providing an affordable point-of-care diagnostic solution for SARS-CoV-2 detection. Using upcoming technologies like three-dimensional (3D) printing that extensively make use of nonthermal plasma processes, devices based on elastomeric polymers like PDMS can be developed with the promise of

![Figure 2](https://example.com/figure2.png)

**Figure 2** Plasma processes: surface activation, grafting, Polymer coating (PECVD/PP), etching, crosslinking and immersion ion implantation, and their applications in different domains of the healthcare industry. PECVD, plasma-enhanced chemical vapor deposition; PP, plasma polymerization.
great savings on time and effort. Fulfilling these criteria of high feasibility and rapid, large-scale screening is essential to curb spread of COVID-19 type diseases, besides providing a clear direction toward our future efforts in fighting similar pandemics.

3.1 Plasma process for fabrication of microfluidics devices

Micropatterning is one of the important steps used to create microchannels during the microfluidics fabrication process. Plasma processing plays an important role in each of the three micropatterning methods: (i) indirect method, (ii) direct method, and (iii) thin-film sacrificial layer method (Figure 3).

Indirect micropatterning (Figure 3a) is a master replica-based method that incorporates a set of techniques, collectively termed as “soft lithography,” for manipulating polymer microstructures. It includes replica molding, microcontact printing, casting, injection molding, and hot embossing processes. Plasma processing is involved in three stages of microfabrication of PDMS polymer-based microfluidic device: Step 1: Master fabrication by plasma etching; Step 2: Fluorination of master surface by plasma activation, grafting, or PECVD; and Step 5: Plasma-induced sealing of PDMS microfluidic device. Using this approach, microstructures with dimensions down to even 10 nm can be easily created on polymers, such as PDMS, without the need for any expensive cleanroom facilities.

The direct micropatterning method (Figure 3b) is based on the controlled etching of the material to produce nanostructural features directly on the substrate. Some examples of direct micropatterning methods include photolithography, electron beam lithography, ion-beam lithography, stereolithography.

**FIGURE 3** Schematics of microfluidics fabrication methods. (a) Indirect micropatterning method: (1) master fabrication by plasma etching, (2) fluorination of master surface by plasma activation, grafting or PECVD, (3) PDMS curing, (4) stripping off micropatterned PDMS from master, and (5) plasma-induced sealing of PDMS microfluidic device; (b) direct micropatterning method: (1) plasma cleaning, (2) photoresist deposition, (3) UV exposure through mask, (4) development of photoresist patterns, (5) plasma etching, (6) stripping/ashing of photoresist, and (7) sealing of PDMS to substrate after oxygen plasma treatment; (c) thin-film sacrificial layer micropatterning process: (1) PECVD of SiO$_2$/SiN$_x$ onto Si/glass substrate, (2) deposition of aluminum layer by plasma sputtering process, (3) deposition of photoresist on to aluminum layer, (4) micropatterning by photolithography, (5) reflowing of photoresist at high temperature, (6) etching of unprotected Al layer, (7) deposition of a thick layer of SiO$_2$/SiN$_x$ by PECVD, (8) etching of sacrificial core. PECVD, plasma-enhanced chemical vapor deposition; PDMS, polydimethylsiloxane.
with focused laser beam lithography, and plasma etching. Although a suitable mask is used to write the microstructures on the substrate, new developments have done away with the need for masks, enabling the writing of micro- or even nano-features directly onto the substrates using techniques, such as scanning electron microscope lithography, focused ion beam lithography, focused laser beam lithography, and so forth. Plasma technology provides a convenient method for micropattern fabrication in mass scale and sealing glass and polymer-based microfluidic devices without using any adhesives. A typical glass or silicon-based microfluidics fabrication requires the use of plasma processes in various fabrication steps, namely plasma cleaning of the substrate, plasma dry etching of substrate for micropatterning, stripping of photoresist by plasma etching, that is, ashing, and plasma treatment for irreversible sealing of microfluidic device.

Recently, some interesting work on the role of plasma processing in the advancement of microfluidics fabrication has been reported. Yadavali et al. have focused on fabricating highly parallelized three-dimensional microfluidics using the PECVD approach. These structures improve the process throughput by enabling simultaneous operation of several replicate devices on a single chip. Plasma treatments, which have been applied to modify the surface properties of polymer particles through surface cleaning, alteration of crystallinity, or through crosslinking and introduction of surface polarity, without changing the bulk composition, have proved extremely useful in 3D printing. Plasma-treated surfaces demonstrate excellent adhesion properties and are therefore suited for preparing 3D-printing parts for bonding. For instance, Pranzo et al. have reported on extrusion-based 3D printing for designing microfluidic devices. The fused filament fabrication-based 3D printers have been used to tailoring devices based on 2D closed channels, a process that utilizes oxygen plasma to improve surface adhesion in silicon-based polymers meant for the fabrication of interconnected modular parts.

3.2 | Plasma processes for performance improvement of microfluidics device

3.2.1 | Improving wettability of microchannel

Microfluidic devices require a hydrophilic surface for aqueous analytes to spread and flow smoothly and consistently through the microchannels to the detection and other processing elements of the device. Hydrophobic polymer microchannels can be surface-modified using plasma processes such as surface oxidation using spatially controlled plasma oxidation treatment, plasma-induced grafting, and PECVD of hydrophilic polymer (e.g., polyacrylic acid, polyvinyl alcohol [PVA]) coatings. Tsougeni et al. have reported the fabrication of PDMS hydrophilic surface for reusable droplet microfluidics via deposition of PVA after oxygen plasma treatment of PDMS surfaces, for efficiently generating monodispersed oil-in-water emulsions. Tsougeni et al. applied mass production-amenable plasma processing technology for fabrication, surface modification, and multifunction integration in “smart” multifunctional polymeric microfluidic devices with controlled wetting, capillary filling, and hydrophobic valving. In another recent development, Gökaltun et al. have blended smart copolymer segments of polyethylene glycol (PEG) and PDMS (PDMS–PEG) with PDMS during device fabrication to retain its hydrophilicity for at least 20 months. This PDMS modification method can be further applied in analytical separations, biosensing, cell studies, and drug-related studies.

Future applications of microfluidic technology, where nanoliter quantities of chemicals are processed on an integrated chip, would gain significantly from the preparation and precise control of small droplets, especially double emulsions that can act as miniaturized confined chemical reactor. Barbier et al. showed a robust way to create stable PDMS surfaces on demand based on plasma polymerization of acrylic acid (PPAA) coatings, deposited on PDMS to reach fully hydrophilic or patterned hydrophobic/hydrophilic microsystems. Such surfaces lead to the opening of large perspectives in many fields and, for example, in two-phase flow systems so as to generate water-in-oil-in-water (W/O/W) double emulsions for the first time in a device made of PDMS. Indeed, plasma crosslinked coatings provide the possibility of running such experiments successfully for at least 3 weeks with no discernible aging of the surface. For comparison, the lifetime of O2 plasma-activated microsystems (30”, plasma cleaner) under the same conditions is less than one day. Both W/O/W and oil-in-water-in-oil double emulsions have been of considerable interest owing to their potential applications in food science, cosmetics and pharmaceutics. Microfluidic devices based on such an approach can prove useful in a variety of fields including analysis of confined chemical reactions, biological screening, drug delivery systems, and control of particle morphology.
3.2.2 | Preventing biofouling of microchannels

PP process has been used to deposit antifouling polymer coatings to restrict nonspecific adsorption of biomolecules like proteins from biological analytes that otherwise can compromise the efficiency, reproducibility, and long-term biomedical application of microfluidic devices. Polymer coatings like PEG and tetraglyme have been successfully developed by plasma processing approaches and demonstrated to impart excellent antifouling properties to microchannel surfaces.\(^{[57]}\) This aspect will be delved upon in greater detail in the next section.

3.2.3 | Facilitating the flow of analyte through microchannel

Flowability of the analyte through the microchannels is one of the important parameters that govern the performance of microfluidic device. This can be achieved either by improving the surface wettability or by facilitating electro-osmotic flow (EOF) through modification of surface properties of the microchannel. Electro-osmosis or electrodynamics is one of the common pumping methods used for uninterrupted flow of biological analyte solution through microfluidics channels. PECVD-deposited SiO\(_2\) and SiN\(_x\)-based microfluidic systems with hydrophilic microchannel surfaces facilitate the flow of analytes by the electro-osmotic pumping process, making the analysis much faster.\(^{[58]}\) Similar to the PECVD process, plasma activation processes such as plasma oxidation or silanization can also influence electro-osmotic properties by altering the zeta potential of the modified material. EOFs are dependent on the surface charge of the material, and the surface charge for polymers can be highly variable depending on the material and the manner in which it is treated. The zeta potential, which is related to diffuse layer charge density, can be modulated by surface treatment of the material. Chai et al.,\(^{[59]}\) for example, made use of DC-pulsed oxygen plasma treatment to modify the surface of PMMA. Zeta potential measurements confirmed an increase in the negative charge for the treated PMMA surface, which followed the trend of increasing hydrophilicity observed with an increase in the plasma treatment duration, and a corresponding enhancement in the streaming current and streaming potential. The same approach of utilizing plasma oxidation to increase zeta potential was adopted by Snyder et al.\(^{[60]}\) to develop ultrathin, porous, nanocrystalline silicon membrane-based electro-osmotic pumps capable of pumping microliter per minute range flow with applied voltages as low as 250 mV.

4 | ANTIBIOFOULING, SELF-DECONTAMINATING, AND BIOMIMETIC SURFACES

Biofouling refers to the process of adhesion and proliferation of undesired biomolecules like proteins, carbohydrates, microorganisms, and cells on material surfaces. The initial step in biofouling is the adhesion of biomolecules present in the fluid, which form a conditioning film for subsequent microorganism adhesion, leading to biofilm formation and bacterial infection.\(^{[61]}\) The conditioning film modifies the surface properties of the bare material surface\(^{[62]}\) and promotes bacterial adhesion to it, besides providing nutrients at the surface.\(^{[63]}\) This can pose severe implications in the case of medical and healthcare devices, such as prosthetic heart valves, catheters used in urinary tract infection and cesarean surgeries, intraocular lenses, breast and bone implants, and so forth, as biofilms once formed are untreatable with conventional antibiotics. Moreover, early impairment of implants necessitates premature removal from the body that not only adds to the cost but also to the patient’s discomfort.\(^{[16,64]}\)

The most common strategy for the prevention of biofouling and microbial infections on MDs is to use antimicrobial agents that kill or inhibit the growth of the microorganism. However, the modern approach focuses on targeting the biomaterial surface–body fluid interface, which represents the hot spot for accidental contamination and clinically relevant infection. In Figure 4, we present the different strategies currently employed for the fabrication of antifouling surfaces for healthcare and biomedical applications.

4.1 | Surface engineering approach using plasma processes

This is a surface free energy (SFE) modulation approach, which works mainly on three strategies, using (i) hydrophilic, (ii) hydrophobic, and (iii) amphiphilic surfaces.

A substantial amount of work has been carried out on this strategy to engineer and develop bioinert surfaces using hydrophilic, high SFE polymers with specific chemical and structural properties.\(^{[65,66]}\) One of the best examples of such polymers is PEG, well known for its antifouling characteristic against proteins and bacteria.\(^{[67,68]}\) PEG molecules are highly mobile in an aqueous environment and attain extremely large hydrodynamic volumes. Compression and steric hindrance of extended PEG brushes by protein/biomolecule adsorption restrict mobility of the polymer chains, leading to unfavorable
entropy loss and making the biomolecule adsorption process entropically nonfeasible. Protein–polymer adsorption enthalpy ($\Delta H_{\text{ads}}$) can be either favorable or unfavorable depending on protein–surface interactions.\cite{69} In addition, the stable interfacial water layer on hydrophilic PEG surfaces acts as a barrier against the interaction of biomolecules with the material surface. Another contributing factor is the minimum interfacial free energy of PEG films with water (free energy of the polymer–water interface). As the energy approaches zero, the driving force for protein adsorption decreases.\cite{70} Accordingly, Lee and Voros\cite{71} have reported plasma-induced grafting of PEG to generate antifouling PDMS surfaces for microfluidic devices. PEG-coated high-density polyethylene and Pyrex tubes, developed via transport discharge in atmospheric plasma, have shown excellent antifouling properties with respect to CT-26 (colon cancer).\cite{72} Recently, Stahel et al.\cite{73} have demonstrated that atmospheric-pressure plasma-polymerized polyoxazoline coatings show remarkable promise as a new class of antibiofouling, biocompatible polymers for biomedical applications. Difficult to synthesize by conventional methods, plasma deposition of 2-methyl-2-oxazoline-based coatings in nitrogen atmospheric-pressure DBD has proved to be a successful route toward synthesizing these films. Moreno-Couranjou et al.\cite{74} work on catechol-based coatings is particularly interesting, as it reports an upscalable atmospheric-pressure plasma deposition method to deposit multicomponent films that exhibit both antibacterial (from immobilized Ag nanoparticles) and antifouling properties (through grafted enzyme dispersine B).\cite{74} Bhatt et al.\cite{75} have developed, for the first time, nano-thick peptidomimetic coatings using a low-pressure inductively excited pulsed plasma polymerization of 2-ethyl-2-oxazoline (ppEtOz) to control the cell–surface interactions for biological applications. The cell-adherent and cell-repellent properties of the deposited ppEtOz coatings were fine-tuned by varying the plasma power and monomer flow rate. The PECVD of natural products, such as 1,8-cineole (a tea tree oil precursor), has also been explored as a possible candidate for hydrophilic antimicrobial coatings and has been demonstrated to be highly effective against biofilm formation.\cite{76}

The low-surface-energy approach is based on designing hydrophobic surfaces with low SFE. Fluorocarbon polymer coatings are an ideal candidate to prevent biofouling in the early stages of biofilm formation by offering an inert, nonstick surface to bacteria and other colonizing microorganisms.\cite{77, 78} This approach was inspired by a well-known natural antifouling surface consisting of gorgonian corals, with SFE in the range of 23–27 mN/m.\cite{79} The “Baier curve” represents the generalized correlation between bioadhesion and the substrate surface energy as a nonlinear curve with a minimum at 20–30 mN/m, which is the optimum SFE range of a substrate for negligible bacterial adhesion.\cite{80} It is interesting to note that, besides minimal bacterial adhesion to low SFE surfaces, it is easy to detach the adhered bacterial cells under physical shear stresses, like flow or passage of air–liquid interface.\cite{81}

Fluorinated plasma discharges\cite{82} like CF$_3$ RF plasma have been used for the hydrophobic treatment of polyethersulfone and nylon membranes to prepare biomimetic membranes for use in biorelevant dissolution studies.\cite{83} Our group has also carried out extensive work on the use of PECVD to develop low SFE nanostructured fluorocarbon-based antibiofouling coatings. Kumar et al.\cite{84} have reported antifouling properties of fluorocarbon coatings fabricated by PECVD of perfluorocarbon precursor 1H,1H,2H,2H-perfluorodecyl acrylate (PFDA) against proteins ovalbumin, human albumin serum, and fibrinogen.\cite{84} Plasma-polymerized hydrophobic-fluorinated coatings have also been deposited on cotton fabric using a DBD atmospheric plasma setup. The PP occurred preferentially on the cotton surface and imparted excellent abrasion and washing

![Diagram](Image_url)
resistance behavior. Such materials have the potential to be employed in the fabrication of antimicrobial and antifouling garments.[85]

Another novel approach adopted by our group was to develop amphiphilic copolymer-based smart and responsive coatings with the capability of undergoing reversible changes in properties in response to surrounding environmental conditions or stimuli. We have reported, for the first time, the application of single step, solvent-free, low-pressure PP process to develop nanostructured amphiphilic coatings with optimized SFE using two noncompatible precursors, that is, hydrophilic diethylene glycol dimethyl ether (DEGDME) and hydrophobic PFDA.[86,87] The PFDA-co-DEGDME coatings exhibited both switching and antibiofouling characteristics. The compositional, topological, and morphological complexities of the amphiphilic surface weaken the hydrophobic or hydrophilic interactions between the biomolecules and the substrate.[88] Furthermore, the improved protein repellent property of amphiphilic PFDA-co-DEGDME coating was also a consequence of the synergistic effect of a well-known protein-resistant PEG-like surface, the inert, anti-adhesive nature of perfluorocarbon coatings, and the compositional, topological, and morphological complexities of the surface.

4.2 | Antimicrobial agents-bound/embedded self-decontaminating surfaces

In this class of biofouling prevention strategy, antimicrobial polymers such as quaternary ammonium salt (QAS) and phosphonium salt (PS)-based polymers and synthetic antibiotics, which work against bacteria, virus, fungus, and so forth, are covalently bound to the surface of the device.[89,90] Antimicrobial activity of QAS and PS groups is derived from ionic and hydrophobic interactions between the QAS/PS and microbial cell wall/cytoplasmic membrane components.[91] Whereas the nonpolar chains of QAS/PS interact with the nonpolar, inner part of cytoplasmic membranes, the positive charges on the groups interact with the polar outer part of the membranes, disrupting the electrical and osmotic balance and other important functions of the cell to culminate in bacteriolysis.[92] QASs have been plasma-grafted on different surfaces, namely polypropylene,[93] glass fiber membranes,[94] and polyethylene foils[95] to impart antibiofouling properties.

Depositing a polymer coating impregnated with antimicrobial agents has also been explored to inactivate microbes attached to the device surface. Hendricks et al. have used PECVD to deposit PEG-like and polybutylmethacrylate coatings embedded with an antibiotic (ciprofloxacin™) on polyurethane surfaces for its controlled release from the surface.[96] Kumar et al.[97] have deposited silver nanoparticle-embedded PPAA on PET meshes for antibiofouling surgical mesh applications. The carboxylic groups of PPAA act as anchors as well as capping and stabilizing agents for the chemically synthesized Ag NPs, demonstrating more than 99% bacterial reduction when compared with the untreated PET mesh.

Antibiofouling and self-decontaminating surfaces can play a vital role in restricting COVID-19 transmission and reuse of PPEs. As the SARS-CoV-2 virus has a protein envelope (S, E, and M proteins) surrounding it, protein repellent surfaces developed by plasma processes can work as effective antiviral surfaces and help prevent the spread of COVID-19 through touch.[98] Recent studies have shown that the virus can remain infective on metal, glass, wood, fabrics, and plastic surfaces from several hours to days. As it is practically impossible to keep surfaces permanently sanitized, the solution lies in introducing an antiviral coating on these surfaces. Nanoparticles like ZnO, CuO, Ag, Cu, and QASs are ideal candidates to achieve this means. QASs, in particular, are known to be highly effective against influenza virus as well as bacterial strains (both Gram-positive and negative).[99]

4.3 | Biomimetic surfaces

The concept of “biomimetics,” a term that refers to the process of imitating or learning from biological systems or natural processes, has been applied to derive unique functional surfaces that can address issues pertaining to biocompatibility and biofouling properties. This holds special relevance in the case of implants. Biomimetic surfaces have found use in orthopedic implants,[100] devices for controlling cardiovascular diseases (stents, catheters, vascular grafts, etc.),[101] dental implants, synthetic organs, and so forth.[102] Given the many different biomedical devices and implants, as well as the different cells, tissues, bacteria, and proteins involved, there is no universal solution to all problems. Hence, cell adhesion, antibacterial, and antifouling properties have to be tailored to each specific need. In the process, plasma treatment has emerged as one of the most attractive methods for tailoring such novel surfaces for niche applications. Table 1 lists some of the major advances in the field of plasma-treated biomimetic surfaces from the last decade. Earlier developments in this field have been discussed elsewhere[113] in greater detail.
In the preceding decade, plasma-deposited coatings have been extensively investigated for controlled delivery of a broad spectrum of drugs in vitro and in vivo. Based on the desired biomedical application, rapid release of a drug may be desirable, for example, in case of rapid pain relief, whereas sustained and significantly slower release are required for hormonal and anticancer therapies. Accordingly, Bhatt et al. reported fine-tuning of PP coatings to obtain different drug release profiles. For instance, on different biologically relevant surfaces, bio-degradable and biocompatible polycaprolactone (PCL)-co-PEG polymer coatings have been developed by gradually varying the partial pressure ratio of the monomers (ε-CL/DEGME) under a low-pressure inductively excited RF plasma reactor.\textsuperscript{114-119} Cell-adherent and cell-repellent properties of various PCL-co-PEG coatings have also been investigated on the plasma-deposited layers. Table 2 illustrates some of the important PP coating-based drug delivery systems that have been developed over the last few years.

In addition to conventional PP coatings, plasma crosslinked hydrogels have also been recently explored as potential drug delivery vehicles for therapeutic applications. Labay et al.\textsuperscript{133} have recently demonstrated how in situ generated RONS in plasma crosslinked alginate demonstrates cytotoxic potential toward bone cancer cells. Nolan et al.\textsuperscript{134} however, have incorporated functional Ag and Au nanoparticles into plasma crosslinked PVA gels and

| S. no. | Polymer functionalization approach | Application/observation | References |
|-------|-----------------------------------|-------------------------|------------|
| 1.    | POSS-PCU functionalization with bone-inducing peptides for tissue engineering applications | Induction of cell adhesion and bone mineralization | Gentile et al.\textsuperscript{103} |
| 2.    | Siloxane and fluorinated siloxane elastomeric coatings deposited using an atmospheric-pressure plasma jet system | Serum protein adsorption and bacterial attachment studies for antimicrobial property investigation | Stallard et al.\textsuperscript{103} |
| 3.    | Radical-functionalized PP films via combination of plasma polymerization and plasma immersion ion implantation techniques | Covalent attachment of fibronectin or bone morphogenetic protein-2 for biofunctionalized coatings in bone implantable devices | Akhavan et al.\textsuperscript{104} |
| 4.    | Acrylic acid functionalization of Ti surface | Immobilization of bioactive arginine-glycine-aspartic acid (RGD) peptide for the acceleration of tissue integration in bone implants | Seo et al.\textsuperscript{105} |
| 5.    | Ion-assisted plasma polymerization on Ti surface using gas mixture: Ar, acetylene, N\textsubscript{2} | Ag nanoparticles immobilization for prevention and treatment of biomaterial-associated infections in orthopedic implants | Akhavan et al.\textsuperscript{106} |
| 6.    | HIPIMS carbon coating on stainless steel cardiovascular stent | Covalent immobilization of cell-adhesive extracellular matrix proteins, tropoelastin, and fibronectin for increased proliferation of endothelial cells | Ganesan et al.\textsuperscript{107} |
| 7.    | PECVD of Ag/amorphous C:H nanocomposite on Ti surface | Introduction of antibacterial performance and biocompatibility in biomedical implants | Thukkaram et al.\textsuperscript{108} |
| 8.    | N\textsubscript{2}-PIII modification of PEEK | Antibacterial property against Staphylococcus aureus. Potential bone tissue engineering and dental applications | Gan et al.\textsuperscript{109} |
| 9.    | Plasma polymerization of cyclopropylamine on poly (ε-caprolactone) nanofiber meshes | Improved cell adhesion and proliferation | Chan et al.\textsuperscript{110} |
| 10.   | Oxygen, argon, and nitrogen plasma treatment of poly (3-hydroxybutyrate-co-3-hydroxyvalerate) film | Improved cell attachment observed with HaCaT cultures | Garrido et al.\textsuperscript{111} |
| 11.   | Oxygen plasma-mediated carboxy functionalization of electrospun poly(lactic acid) nanofibers | Grafting of cationized gelatin for enhanced viability, proliferation, and differentiation of rabbit articular chondrocytes | Chen et al.\textsuperscript{112} |

Abbreviations: HIPIMS, high-power impulse magnetron sputtering; PECVD, plasma-enhanced chemical vapor deposition; PEEK, polyether ether ketone; POSS-PCU, polyhedral oligomeric silsesquioxane-poly(carbonate-urea) urethane.
studied their antibacterial properties. Gel-based drug delivery systems allow local confinement and efficient delivery of the drug to the affected site.

6 | BIOSENSORS

A biosensor is an analytical device that makes use of biological reactions to detect target analytes in the presence of other unwanted interfering moieties. The most common applications of biosensors include glucose monitoring in diabetes patients, detection of pathogens and pollutants, determining levels of toxic substances before and after bioremediation, and determination of drug residues in food (antibiotics and growth promoters). The performance of a biosensor largely depends on how well the bioreceptor components are attached to the surface of biorecognition unit, which is known as “interfacial design” or “biomolecular immobilization.” A general immobilization approach involves precoating the biorecognition unit surface with polymer films capable of immobilizing bioreceptors (e.g., antibodies). However, certain problems associated with conventional copolymer coating remain unresolved, such as weak adhesion to the substrate, low mechanical strength and chemical stability, and nonhomogeneity of the coating.

Based on their transduction modes and recognition elements, biosensors may be categorized into (i) electrochemical biosensor, (ii) label-free (surface plasmon resonance [SPR] or quartz crystal microbalance [QCM])-based biosensors, and (iii) label-based (radioisotope, fluorophore, ELISA) biosensors.  

| S. no. | Polymer film | Drug loaded (status) | References |
|-------|--------------|----------------------|------------|
| 1.    | PCL-co-PEG   | Simvastatinacid (orthopedic applications) | Canal et al[117] |
| 2.    | Multilayered PCL-co-PEG | (i) Cis-platin (in vitro and in vivo for apoptosis of ovarian cancer cells) (ii) Carboplatin (preclinical studies on ovarian [OVCAR-3NIH] and colon [CT26] cancer cell lines) | Bhatt et al.[120] Dybiat et al.[121] |
| 3.    | Plasma-polymerized n-butyl methacrylate | Ciprofloxacin (zero-order kinetics observed in vitro) | Yuan et al.[122] |
| 4.    | n-Heptylamine coatings | Levofoxacin (inhibition of methicillin-resistant Staphylococcus aureus colonization and biofilm formation) | Vasilev et al.[123] |
| 5.    | Poly (lactic-co-glycolic acid) | Fluorescein diacetate (6-fold-high biodhesion with cumulative drug release in vitro) | Mogal et al.[124] |
| 6.    | Polyacrylic acid | Doxorubicin[124] (reduced release rate through thickness control of plasma deposition) | Myung et al.[126] |
| 7.    | Nylon 6,6, | Ampicillin (antibacterial effect against Staphylococcus epidermidis studied through tailoring reservoir film thickness and release kinetics) | Kratochvil et al.[125] |
| 8.    | Poly(tetramethylcylo-tetrasiloxane) | Daunomycin, rapamycin, and NPC-15199 (thromboresistance and biocompatibility of coatings in vitro) | Osaki et al.[127] |
| 9.    | PTFE | Camphothecin (correlation of drug release profile with plasma polymer coating times) | McInnes et al.[128] |
| 10.   | PLA-co-polyester urethane | Temozolomide (TMZ) (reduction in undesirable burst effect and improved release rate of TMZ) | Stloukalet al.[129] |
| 11.   | Polyallylamine | Vancomycin (controlled porosity of polymer film to fine-tune release of hydrophilic drug) | Simovicet al.[130] |
| 12.   | Plasma-polymerized PEG-like coatings on β-TCP ceramic | Gentamicin and ampicillin (antibiotic-releasing bioceramics for eradication of S. aureus) | Khurana et al.[131] |
| 13.   | Plasma-polymerized sulfur hexafluoride (SF6) coating | Ampicillin (eradication of S. aureus and Escherichia coli using drug diffusion test in vitro) | Meydan et al.[132] |

Abbreviations: β-TCP, β-tricalcium phosphate; PCL-co-PEG, polycaprolactone-co-polyethylene glycol; PLA, poly(lactic acid); PTFE, polytetrafluoroethylene.
6.1 Plasma process for electrochemical biosensor

An electrochemical biosensor comprises of enzymes immobilized on electrochemical transducers such as metal, carbon, glass, or ion-selective electrodes. For efficient sensor performance, the distance between the electrode and catalytic enzyme is to be kept at minimum and the oxidizable interfering species (e.g., ascorbic acid and uric acid) should not infiltrate the interface layer. Therefore, the interface layer in a biorecognition unit should not only be thin but also possess optimized bulk porosity to avoid diffusion of interferents. Thin functional films obtained by PECVD tick both these boxes.

Commercialized biosensors used for monitoring daily sugar levels by diabetes mellitus patients employ advanced mediator–enzyme biosensors.[138] Here, the enzyme and mediators are embedded or immobilized in/onto a cast polymer on an electrode surface. The mediator–enzyme biosensors work on the principle of electron mediators (e.g., ferrocene) mediating the electron transfer between the catalytic center of the enzyme, i.e. Glucose Oxidase (GOx) and the electrode. The effective electronic signal, which is proportional to the glucose concentration, remains unaffected by the oxygen concentration or presence of interferents. PECVD and plasma activation have been successfully used to fabricate electron transfer-mediated biosensors with a high degree of reproducibility and adhesion to the electrode.[139, 140] Working on similar lines, Arefi-Khonsari’s group have reported the use of low-pressure plasma to generate a stable allylamine coating on carbon surface electrodes and subsequent covalent immobilization of enzyme laccase.[141] In a further advancement to this technique, Liu et al.[142] have recently used a single-step aerosol-assisted DBD atmospheric-pressure plasma for simultaneous deposition of an enzyme-embedded plasma-polymerized ethylene film (instead of the conventional multistep process) to fabricate efficient biosensor systems. The process is much faster than conventional approaches and demonstrated immunostaining results close to that of covalently bonded protein.

FIGURE 5 Schematics of fabrication of (a) a typical SPR biosensor and its working principle and (b) DNA biosensor using PPFs (A) PAA and (B) PGMA. PAA, poly acrylic acid; PGMA, poly (glycidyl methacrylate); PPF, plasma polymerized film; SPFS, surface plasmon field-enhanced fluorescence spectroscopy; SPR, surface plasmon resonance.
6.2 Plasma process for SPR-based biosensors

SPR detection for monitoring biomolecular interactions, for example, antigen–antibody, receptor–ligand, and nucleic acid base pair association, has been widely used as a label-free, real-time measurement tool for biosensor applications. Figure 5a depicts the schematics of a typical SPR biosensor with different components and its working principle. A number of SPR biosensors have been commercialized over the years including BIAcore 4000, SPRi-plex II, SensIQ pioneer, and so forth, which have shown tremendous promise in efficient and real-time measurements. Recently, Qiu et al. have been successful in developing a dual-functional SPR biosensor with highly sensitive, fast, and reliable diagnostic capability for SARS-CoV-2 virus detection. Their detection technique is based on the localized SPR of tiny immobilized Au nano-islands that are functionalized with DNA receptors specific to the SARS-CoV-2 RNA sequences. Validation tests have proved successful with the closely related SARS-CoV virus, which was responsible for the 2003 SARS pandemic, proving that the method is fast, accurate, and highly selective.

PECVD is an ideal process to deposit very stable, ultrathin (<200 nm) polymer films with desired functional groups, like carboxylic and amine groups, for immobilization of bioreceptors to the device surface either via electrostatic interaction or covalent bonding. Manickam et al. have used PECVD to deposit SiOx-CyH2 layer, using tetraethyl orthosilicate precursor, on silver thin films and demonstrated its successful application in mouse Ig immunoassays. Wijaya et al. have provided a detailed account of various plasma-based techniques applied in SPR biosensor design strategies. Considering the advantages, there is ample scope for extending the plasma processes into developing high-performance SPR biosensors targeted toward the detection of SARS-CoV-2 and other similar viruses in the near future.

6.3 Plasma process for QCM-based biosensor

QCM is a label-free, easy-to-use, and highly sensitive piezoelectric biosensor. QCMs can be used to detect the mass change associated with a biospecific binding event, usually antigen–antibody binding or complementary DNA hybridization. As covalent immobilization methods for introducing any biological recognition element that is based on crosslinking reagents, such as glutaraldehyde (GLU), carbodiimide, succinimide, and so forth, suffer from limitations in terms of steric hindrance, decreased antigen-binding efficiency, and detection sensitivity, the focus has shifted to plasma processing technology for innovative interfacial design strategies.

Plasma processing technology coupled with nanotechnology ensures high loading capacity and bioactivity of immobilized biomolecules, along with enhanced sensitivity and reusability. Li et al. employed an SF6 + He ICP to fabricate lead-free piezoelectric biosensor as a viable platform for nucleic acid testing. Makhneva et al. however, have used PP to develop cyclopropylamine-based effective QCM biosensors. In another approach, Khusnah et al. resorted to low-pressure oxygen plasma treatment of polystyrene-coated QCM biosensors to alter the surface hydrophobicity and improve the immobilization rate of the biomolecule to the sensor surface. Thus, plasma technology in all its forms has played a significant role in the performance enhancement of QCM biosensors.

6.4 Plasma processing for DNA sensors

DNA sensors and oligonucleotide probe array, or “DNA chips” are attractive due to their wide applications in extracting genetic information from viruses or bacteria, diagnosis of disease, as well as for environment monitoring. Through prudent selection of precursors and plasma parameters, a variety of functional groups, for example, amine, carboxyl, epoxy, etc., can be incorporated in deposited plasma polymer films and utilized for immobilization of DNA probes. Arefi-Khonsari’s group has reported the PECVD of acrylic acid (AA) for tailoring DNA chips via covalent immobilization of biomolecules on glass substrates. Figure 5b illustrates two different approaches in which plasma can be used for designing DNA biosensors. Figure 5b(A) depicts the PECVD of AA on a glass substrate, followed by probe coupling using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide/N-hydroxysuccinimide in a Biorobotics Microgrid II instrument (Genomic Solutions). The final step is the hybridization of the microarrays with fluorescent Cy3-labeled complementary probe targets. The alternate approach (Figure 5b(B)) involves PECVD of glycidylmethacrylate (GMA) film on a gold substrate, followed by DNA probe immobilization. The use of GMA offers benefits such as covalent immobilization of DNA probe via a facile epoxide–amine coupling reaction, which does not require any activation step, and lesser chances of spreading of the solution due to the relatively hydrophobic nature of GMA. Moreover, these DNA sensors exhibit good resistance...
to nonspecific DNA adsorption arising from the hydrophobic characteristics of plasma-polymerized poly (GMA) surfaces.

Vermisoglou et al.\textsuperscript{[156]} have, in their recent review, extensively discussed the role of various graphene-based DNA biosensors in the detection of several viruses including SARS-CoV-2. For SARS-CoV-2 virus, which consists of a single RNA strand, biosensor kits based on real-time reverse transcriptase PCR (RT-PCR) assays have been made available to keep track of the COVID-19 outbreak. The three genes that are currently being targeted are the Orf1 gene (human RNA polymerase protein), the N-gene (the nucleocapsid protein), and the E-gene (envelope protein).\textsuperscript{[157]} Another latest innovation of note is the clustered regularly interspaced short palindromic repeats (CRISPR)-based diagnostic tool. Sherlock Biosciences has used this technology to create a DNA endonuclease-targeted CRISPR trans reporter platform capable of detecting multiple coronavirus strains. The full assay can be run in only 30 min and is able to distinguish SARS-CoV-2 with no cross-reactivity for related coronavirus strains, with a sensitivity comparable to conventional methods. Plasma technology can play its part in this exercise by facilitating the design of effective DNA biosensors for low-cost mass testing and with better efficiency in terms of sensitivity and time of detection.

### 6.5 Plasma process for ELISA

ELISA is an immunological assay-based gold standard method, which is commonly used to detect a range of target molecules (e.g., antibodies, antigens, proteins, and glycoproteins) in biological samples, using appropriate partner molecules. Briefly, the binding of the target (antigen) and the probe (antibody) on the ELISA plate is detected by a detection antibody, labeled with an enzyme (e.g., horseradish peroxidase, alkaline phosphatase), which reacts with the chromogenic substrates to generate colored products that can be measured using a plate reader. Multiple samples can be analyzed in a single ELISA experiment using 96-well plates with high sensitivity and selectivity, even in human crude samples (serum, urine, and saliva).

Membrane-based environmental enzyme immunoassays are useful for qualitative or semi-quantitative screening of samples.\textsuperscript{[158]} Generally, these functions were not used for direct binding of antibodies but required additional bifunctional and crosslinking agents for oriented immobilization. The covalent immobilization of antibodies using crosslinking agents, such as GLU, might restrict the rotational freedom of antibodies and compromise on the immunoactive sites.\textsuperscript{[159]} Rejb et al.\textsuperscript{[17]} employed plasma activation process to functionalize nitrocellulose membranes with amine groups using NH\textsubscript{3} and NH\textsubscript{3}/H\textsubscript{2} plasmas for immobilization of antibodies. Here, antibodies of isoproturon (3-(4-isopropylphenyl)-1,1-dimethyleurea), a commonly used herbicide in Europe, were immobilized through their oxidized oligosaccharide moieties on amine-functionalized nitrocellulose membranes. The activity of the immobilized antibodies was tested using an enzyme conjugate of isoproturon according to an ELISA (Figure 6).

These rapid, multianalyte techniques, with the capability for automation to increase throughput, have emerged as potent weapons for screening COVID-19 cases. The determination of SARS-CoV-2 exposure depends on the detection of either IgM or IgG antibodies specific for various viral antigens including the spike glycoprotein (S1 and S2 subunits, receptor-binding domain) and nucleocapsid protein. Therefore, ELISAs that make use of viral protein coatings to bind specifically to a patient’s antiviral antibodies, generating either a colorimetric or fluorescent readout, are handy for point-of-care determinations. Bio-rad’s “Platelia SARS-CoV-2 Total Ab assay” is an example of ELISA-based technology that has been successfully commercialized for SARS-CoV-2 sensing.\textsuperscript{[160]} It is a semi-quantitative in vitro diagnostic test, in a one-step antigen capture format, for the detection of IgM/IgA/IgG antibodies to the SARS-CoV-2 in human serum and plasma.

### 7 Plasma Sterilization and Decontamination

Sterilization is defined as any process that effectively inactivates, kills or eliminates almost all types of microorganisms, including fungi, bacteria, viruses, spores, etc., from a product without affecting its operational functionality and physical integrity. Contamination of biomaterial surfaces during surgery is the primary cause of implant-related infections,\textsuperscript{[161]} which makes it mandatory for all implants to be sterilized before in vivo use. Moreover, PPE used by health workers and other supporting staff requires proper decontamination to prevent transmission of the viral disease. With the sudden scarcity of PPE globally due to a steep surge in numbers of COVID-19-infected patients, rapid and efficient sterilization of new PPE kits has become indispensable to meet the ever-increasing demands. Moreover, the reusability of PPE kits is another strategy that is being seriously considered by regulatory agencies in the event of a “worst case scenario” situation.
Sterilization methods include autoclaving, ethylene oxide treatment, hydrogen peroxide vapors, ultraviolet germicidal irradiation, gamma-ray irradiation, and electron beam irradiation. However, these methods suffer from limitations in some form or the other. For instance, oven or autoclave sterilization processes are unsuitable for thermosensitive polymeric materials due to their limited thermal stability. The ethylene oxide sterilization method is constrained by long process times and the possibility of residual toxicity in the sterilized materials. Gamma and electron beam irradiation, though fast and highly effective, suffer from radiation-induced degradation of material and the functional properties of products (e.g., N95 Filtration Facepiece Respirators [FFRs]) depending on the polymers used, high capital cost, and stringent radiation safety prerequisites. Nonthermal cold plasma-based decontamination method does not suffer from the above issues, and, therefore, has emerged as a comparatively environment-friendly and inexpensive alternative.[20,162] Sterility, in particular for MDs, is defined in terms of a sterility assurance level (SAL), which is the probability of finding one single nonsterile MD in a batch of 1 million similar devices subjected to the sterilization process. In terms of operational requirements, it is reflected in a reduction of (at least) 6-log_{10} in the number of the most resistant microorganisms with a first-order kinetics. To satisfy the SAL, plasma processes show two or three kinetic inactivation processes. However, achieving a high-level disinfection is possible by designing adequately optimized plasma processes.[161]

Cold plasma has been reported to efficiently combat resistant microorganisms (antibiotic-resistant bacteria, spores, biofilms, fungi), tumors, and viruses, leading to a great deal of interest in harnessing the benefits of this technology.[163-165] The Center for Disease Control and Prevention (CDC), USA, in its recent report, has cited the application of H_{2}O_{2} vapor plasma in the sterilization of FFRs, including N95 masks with the potential for high-throughput production.[166,167] Tuttnauer is a global company that has successfully demonstrated that low-temperature H_{2}O_{2}-based plasma sterilizers can be used to sterilize N95 FFRs.[168] STERRAD 100NX is another H_{2}O_{2}-based nonthermal plasma sterilization system, which is available commercially for rapid sterilization of medical instrumentation.[169] Similarly, AbTox Plazlyte is a commercial setup in which peracetic acid (CH_{3}-COO-OH) is used.[170] In these H_{2}O_{2}/peracetic acid vapor-based plasma sterilizers, the role of plasma is not only to generate reactive species (RONS) that deactivate the pathogens but also to remove/decomposed the unused H_{2}O_{2}/peracetic acid from the chamber. Figure 7 illustrates the schematics of a typical H_{2}O_{2}-based plasma sterilization process and mechanism of pathogenic cell death/inactivation.

Recent progress in plasma science has realized their operation in open air without a vacuum chamber and expensive instrumentation. CAP generates RONS species that exhibit high antimicrobial efficacy against vegetative[7] biofilm-forming microbes on the surfaces[5,6,10] and within endoscope channels.[171] The technique has been reported for successful inactivation of airborne...
microbial agents, food-borne microbes, and infected wounds. To improve bacterial susceptibility to antibiotics, the short treatment of nonthermal plasma has been deployed. Nonthermal argon gas plasma arranged in gastrointestinal endoscopes has been Food and Drug Administration-approved for blood coagulation. Under Phase II clinical trials on 36 chronic wound patients, Isbary et al. found significant reduction (34%; $p < 10^{-6}$) of bacterial load in treated wounds with 5-min daily treatment using nonthermal plasma, an approach that has been proven safe to humans without any side effects. In another successful clinical trial on 24 chronic wound patients with 2-min nonthermal argon plasma (14 with MicroPlaSter alpha device, 10 with MicroPlaSter beta device), the treatment was found effective in eradicating bacterial load from wounds. Under the title of BIOPLASMA Cell Modulator (developed by Photo Bio Care), clinical trials were conducted using CAP (air plasma) treatment for acne and esthetic skin improvement on 31 volunteers with acne. They were treated once per week for 6 weeks, with results of significant improvement of around 75% reduction in acne number score without any significant damage to the skin. The 5-min nonthermal argon plasma treatment has been employed with oral antibiotics synergistically to treat a 49-year-old patient with acquired cholesteatoma of the left tympanic cavity whose external auditory canal was infected with extended-spectrum β-lactamase-producing Escherichia coli, Proteus mirabilis, and Enterococcus faecalis. Various nonthermal plasma sources have thus far been developed for applications in dentistry and oncology.

In the case of viruses such as SARS-COV-2, the strains of respiratory viruses remain stable within the aerosols in air and can infect humans and animals, which is the reason air sterilization is a vital step for inactivation of such deadly and highly infectious viruses. To overcome the potential threat from aerosolized viruses, studies on aerosolized MS2 bacteriophages have been carried out. Complete inactivation of aerosolized MS2 bacteriophages was reported for 120–250 ms CAP treatment. Similarly, SARS-COV-2 virus in aerosols can be also inactivated by CAP treatment of air. However, so far, no plasma-based systems are developed and marketed for ventilator incorporated antiviral treatments for COVID-19-like infections without any physiological side effects. In their recent review, Filipic et al. have compiled a broad spectrum of work carried out over the past few years in the field of cold plasma applications for virus inactivation, including the probable mechanisms involved. Table 3 compiles a select list of plasma techniques that have been explored so far for inactivation of viruses.

Virus inactivation is also the first and most important step in vaccine preparation for the prevention of their spread. To prevent degeneration of immunogenicity, virus inactivation must be done within a very short period of time for which CAP has been identified as an efficient alternative. Recently, Newcastle disease virus (LaSota) strain and H9N2 avian influenza virus (A/Chicken/Hebei/WD/98) were inactivated for preparation of vaccine using argon premix with oxygen and nitrogen plasma jet at atmospheric pressure within exposure of 2 min without degeneration of antigenic determinants. There is, therefore, ample scope to investigate the possibility of adopting the plasma inactivation route to develop a suitable vaccine against COVID-19 infection, a possible breakthrough that would be monumental under the current circumstances.

8 | FUTURE PROSPECTS OF PLASMA PROCESSING IN HEALTHCARE: THE ROAD AHEAD

With the benefits of plasma technology well established, it is expected that plasma processes will expand into previously untapped markets and, in particular, make an
A host of techniques have flooded the global market recently in an effort to timely diagnose and identify COVID-19 patients. These comprise of different molecular assays such as RT-PCR, isothermal nucleic acid amplification, nucleic acid hybridization using microarray; serological/immunological assays including ELISA, lateral flow immunoassay, neutralization test; as well as several novel biosensors, including the CANARY biosensor recently developed by PathSensors Inc.\textsuperscript{[198]} Most of these platforms require the use of functionalized, highly specific, and customizable surfaces for immobilization of antibodies, enzymes, DNA, signal amplification moieties, and so forth. Plasma technology offers ease of upscalability and the ability to deliver tailored surfaces with high precision and uniformity. It, therefore, has the potential to contribute significantly toward design of futuristic assay technologies with even greater accuracy and promptitude, and with the promise of being cost-effective, point-of-care test kits capable of mass-scale applications.

With smart PPEs rapidly becoming a reality to ensure enhanced worker safety, plasma technology has the potential to play a significant role in this field. Plasma etching or polymer deposition/grafting processes can be used to design environmental sensors that are embedded in clothing to monitor microorganisms, gas, chemical, sound, UV, heat, impact, and so forth. These sensors alert the wearer in the event of an emergency and thereby help avert any injuries or calamities. India is already using a Mobile app “Aarogya Setu” developed by the National Informatics Centre, Government of India, to control COVID-19 spread with the help of features such as contact tracing, syndromic mapping, and self-assessment. Similarly, many smart electronic gadgets are already available in market, such as smart health bands for online monitoring of health activities, including blood pressure, heart rate, calorie burn, etc. All these smart gadgets and Artificial Intelligence (AI) software can be integrated with PPEs to make them much more interactive and user-friendly.

PP has been employed to tailor a range of novel wound-dressing materials that can promote re-epithelialization to reduce the burden of chronic wounds.\textsuperscript{[199]} Significant research in plasma biology and plasma medicine has revealed that CAP can aid in wound healing by inactivating a broad spectrum of microorganisms and promoting tissue regeneration. This approach can herald a new method toward

### Table 3: Inactivation of wide range of viruses by plasma treatment systems having different composition and geometries

| S. no. | Plasma condition          | Target virus                                      | References                  |
|--------|---------------------------|---------------------------------------------------|-----------------------------|
| 1      | CAP microjet exposure     | MS2                                               | Wu et al.\textsuperscript{[12]} |
| 2      | Surface DBD               | MS2, T4 and Φ174                                  | Machala et al.\textsuperscript{[184]} |
| 3      | DBD plasma torch          | Feline calicivirus (FCV)                          | Yamashiro et al.\textsuperscript{[185]} |
| 4      | CAP-generated short-lived ROS (1O2) | FCV (T) and bacteriophage T4                  | Guo et al.\textsuperscript{[186]} |
| 5      | H2O2 plasma               | Respiratory syncytial virus and influenza A virus | Sakudo et al.\textsuperscript{[187,188]} |
| 6      | O3 plasma                 | FCV                                               | Aboubakr et al.\textsuperscript{[189]} |
|        |                            | MS2                                               | Xia et al.\textsuperscript{[181]} |
|        |                            | Adenoviruses                                      | Zimmermann et al.\textsuperscript{[190]} |
| 7      | NOx plasma (ROS-generating) | Newcastle disease virus                           | Su et al.\textsuperscript{[191]} |
| 8      | Oxygen and nitrogen plasma jet | Herpes simplex virus type1 (HSV-1)                 | Bunz et al.\textsuperscript{[192]} |
|        |                            | Double-stranded DNA, single-stranded DNA, and RNA viruses | Guo et al.\textsuperscript{[186]} |
|        |                            | Inhibition of HIV-1 in macrophages                | Volotskova et al.\textsuperscript{[193]} |
| 9      | RONS-generating plasma    | Potato viral pathogen, potato virus Y             | Filipic et al.\textsuperscript{[184]} |
|        |                            | Tobacco mosaic virus                              | Mehle et al.\textsuperscript{[195]} |
|        |                            |                                                   | Hanbal et al.\textsuperscript{[196]} |

Abbreviations: CAP, cold atmospheric-pressure plasmas; DBD, dielectric barrier discharge; RONS, reactive oxygen and reactive nitrogen species; ROS, reactive oxygen species.
wound healing, particularly chronic wounds and dermatological processes, such as plastic and esthetic surgeries.\[200\]

- Plasma oncotherapy is another interesting prospect that is currently being explored. CAP jets have, for instance, been recently demonstrated to be self-adaptive toward cancer cells and can be fine-tuned to maximize selectivity during plasma treatment.\[201\] Plasma-activated medium (PAM) is another interesting new proposition, wherein a CAP-activated medium, comprising of RNS and ROS, acts as a therapeutic agent with antitumor effects.\[202\] A recent study by Bhatt et al.\[203\] have also shown that novel plasma-activated solutions (PAS) can be used for antimicrobial efficacy against bloodstream catheter-associated multidrug-resistant infections and recurrent episodes of pathogenesis. PAS or PAM can, therefore, be an effective alternative to corrosive and toxic disinfectants, which are currently in use for sanitization and PPE disinfections.

9 | CONCLUSION

Plasma technology has proved to be a successful tool for the valorization of materials and processes through its economic viability, solvent-free, dry, and environmentally benign applicability, and ease of upscaling. The excellent process capabilities of plasma processing have benefited domains such as microfluidics, LOCs, biosensors, antifouling MDs, and controlled drug delivery systems. With significant advantages over conventional fabrication strategies, it can play a crucial role in tailoring robust, low cost, high-throughput devices, and assay techniques to not only combat the present COVID-19 crisis but any future viral pandemic outbreaks also. In addition, direct and indirect CAP technology can be judiciously employed for virus inactivation, sterilization of surfaces, surrounding air, and PPE kits, including N95 FFRLs. Thus, with the rapid strides being made by plasma technology in diverse areas of science and technology, it can be envisaged that plasma applications will continue to see an upward trend in the years to come and open new avenues for technological advancement and scientific innovations that have a positive impact on mankind.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

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