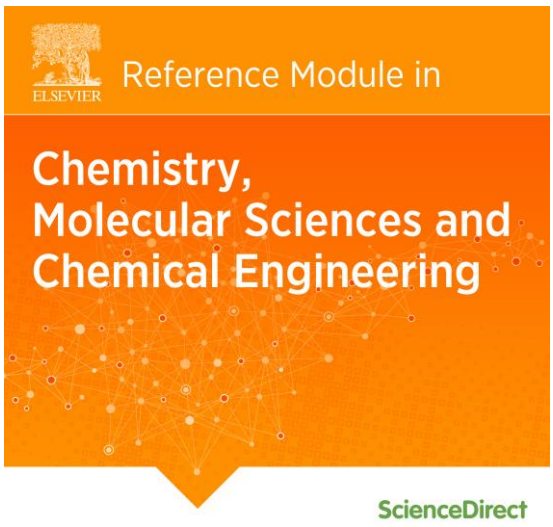

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Plasma Processes for Life Sciences

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Historical Background of Plasma Science and Technology

According to the definition of I. Langmuir plasma is ionized gas matter with equal density of positive and negative particles.[1] Derived from the Greek πλασσειν (to mold), the word plasma identifies "the fourth state of matter," the most common form of visible matter in the Universe. The core of the stars is made of thermonuclear plasma, characterized by nuclear particles and by temperatures of 10^6–10^7 K. Less energetic plasmas are populated by electrons, ions, atoms and molecules in different states; flames, lightning, the solar corona are examples of thermal (equilibrium) plasmas, with quasi homogeneous distribution of energy among the degrees of freedom of the species, and a gas temperature of 10^3–10^5 K. Solar wind, ionosphere and *Aurora Borealis* are cold (nonequilibrium) plasmas; here the energy is stored mostly in the electrons, that remain "hot" at 10^4–10^5 K. The electronic, rotational, and vibrational states of the other species reach lower temperatures, while the translational motion of the neutral species remains "cold" at room temperature (RT). The energy distribution among the various species as well as the distribution of the species themselves depend on many parameters, including the power and the frequency of the electromagnetic field, the pressure and the chemical nature of the gas. Due to de-excitation processes, plasmas typically emit Vis-UV radiations.

Plasma is very common in nature; beside flames, though, men had to wait the 19th century to create plasmas in a controlled environment. Late in that century, when vacuum technology and electricity became mature enough, W. Crookes applied voltage

across electrodes in glass tubes filled with various gases at low pressure (LP), and started to investigate composition and radiation emission of electrical gas discharges. Around the beginning of the 20th century, Crooks tubes were used to generate UV-Vis radiations from LP plasmas and to investigate the structure of the atoms. In the mid-19th century W. von Siemens had already developed "the ozonizer,"[2] a plasma device capable of synthesizing ozone from molecular oxygen at atmospheric pressure (AP), an invention on which paper industry and water treatment plants rely still today, for one of the largest industrial applications of plasma-chemical technology. Plasma Science and Technology (PST) started in those early days, and continues to advance today, with scientific advancements and newer applications.

Widely employed for a steadily increasing number of academic researches and commercial products, Plasma Technology is certainly one of the most established, pervasive, yet still promising technologies of our time. The International Thermonuclear Experimental Reactor (ITER) is expected to succeed in confining and controlling a thermonuclear plasma within the next decade, for a decisive advance toward the virtually infinite production of clean energy for civil use. Thermal plasmas are already used for high temperature applications in metallurgy, welding, metal cutting and waste abatement, as well as in the production of refractory layers and biocompatible hydroxyapatite (HA) ceramic coatings on orthopedic and dental implants. Cold plasmas today are the core technology for the production of UV lights, plasma TVs, integrated circuits in microelectronics, and solar cells, and offer continuously newer applications for advanced materials, communications, energy, environment, food, health and transport. Technological developments have led to the ignition of nonequilibrium plasmas also in liquids largely extending the application fields of plasma processing. And many possible other examples could be listed to highlight the potential of PST for the next decades.[3–5]

Describing all possible examples and applications of plasmas is out of the purpose of this review; here we would like to describe, also according to our experience, how Science and Technology of nonequilibrium plasma impacts Life Sciences today with established examples and potential applications.

Science and Technology of Nonequilibrium Plasmas

Non equilibrium, cold, plasma discharges can be ignited at low (usually 1–100 Pa) or at AP by applying a proper electrical field to a flowing or static gas/vapor mixture in plasma reactors with a suitable configuration. Ionizations, excitations, and breaking of chemical bonds occur, that form active species such as atoms, radical, molecules and ions in ground and excited levels, free electrons, and Vis-UV photons. These species can be utilized in several ways in many existing or proposed technological applications.

Nonequilibrium plasmas became interesting for producing UV-Vis light at the beginning of the 20th century; several kinds of plasma lamps were developed since then. The most important technological applications of cold plasmas, though, blossomed at the beginning of the 1970s due to their ability to tailor the surface properties of materials by means of etching, deposition, and treatment processes. This has generated a market worth hundreds billion of US dollars around several products; computer processors, for example, are so advanced today mostly for the ability of sophisticated plasma etching processes, aided by photolithographic techniques, to "sculpt" 3-D submicrometric features within semiconductors wafers, that lead to powerful and miniaturized integrated circuits.[6] An inventory of several applications of Plasma Science and Technology evolved through the years is listed in Table 1.

Most applications of nonequilibrium plasmas require that the gas temperature remains the closest possible to RT. Since the low efficiency and the low number of elastic collisions at LP limit the energy transfer from the free electrons to heavier species, it is relatively easy to produce cold LP gas discharges. With increasing pressure, however, the electron-species collision frequency increases and the energy transfer becomes more efficient, resulting in gas heating and plasma instabilities such as sparks and arcs. Several approaches can be used to keep the gas cold when the pressure is increased, as in AP discharges, namely: sharp electrodes as in corona discharges; pulsing the plasma with fast rise time and micro-to-nanoseconds wide pulses; improved heat transfer; use of gases (e.g., He) with high thermal conductivity; confining the plasma to submillimiter dimensions, as in microdischarges; and reduce the current with dielectric layers on the electrodes, as in dielectric barrier discharges (DBD).[7,8]

From square meters large deposition plasmas for Solar Cells to microdischarges in plasma TVs, from a few Pa in Microelectronics to 10^7 Pa in certain HID lights, from noble gases in lamps to halogenated gases in etching processes, size, pressure and feed chemistry of cold plasmas span on wide ranges depending on the application, and this poses theoretical and experimental challenges for designing effective plasma sources and reactors for each developed process and application.

Surface Modification Cold Plasma Processes

With respect to other methods in Materials Science and Technology, plasma processes have the advantage of tailoring the surface composition and properties of materials for a submicron depth, leaving intact their bulk features, with dry processes and no need of solvents or liquid-phase reactions. This particular feature makes cold PST processes highly friendly with the environment. Another feature of plasmas, appealing for biomedical applications, is their intrinsic sterility.

AC rather than DC electric fields are used in the KHz (audio frequency, AF), MHz (radio frequency, RF), and GHz (micro waves, MW) ranges to ignite cold plasmas; the field can be applied continuously or in pulsed mode to electrodes, coils in contact with the gas, or trough MW applicators. Main external parameters to be optimized in plasma processes are: geometry of the reactor, chemical

Table 1 Main technological applications of cold plasmas

Gas phase reactions
Production of ozone; abatement of pollutants; removal of CO_2; removal of volatile organic compounds (VOC); treatments of exhaust gas from vehicles and plants
Light sources
Neon lights; high-intensity discharge (HID) car lights; TV plasma displays
Aerospace
Plasma-based space propulsion technologies; plasma-aided combustion; plasma actuators for airplane wings
Surface modification processes of materials
Etching processes
Si, SiO_2 and other materials in microelectronics for ultra large scale integrated circuits (IC); constituent materials of micro electro mechanical systems; polymers for lab-on-chip applications
Deposition of thin films
Gas/vapor barrier layers for food packaging; anticorrosion coatings; photovoltaic coatings; functionalization of very large area substrates (polymer webs, textiles, displays); diamond and diamond-like hard coatings
Treatments
Improved hydrophilicity and cyto-compatibility of polystirene cell-culture plates; grafting of polar groups on polymers for printing; plasma-cleaning of surfaces; improved fiber/matrix adhesion in composite materials
Plasma sterilization of materials
Decontamination/sterilization of surgical tools
Plasma synthesis/functionalization of micro/nanoparticles
Synthesis of semiconductor nano-crystals; synthesis of carbon nanotubes; functionalization of micro/nano-particles
Therapeutic uses of plasmas (plasma medicine)
Plasma-aided surgery; wound healing; cancer treatments; disinfection of teeth cavities in dentistry

composition of the feed; pressure, frequency, power input and on/off modulation (duty cycle) of the electric field, position of the substrate with respect to the plasma, temperature of the substrate, electrical bias potential (for LP processes) of the substrate. External parameters, that can be varied by the operator, actually drive the internal parameters of the processes, for example, the density and distribution of the various species in the plasma, the distribution energy of the electrons, the bias-driven positive-ion bombardment of the substrates (for LP processes), and the overall rate of homogeneous and heterogeneous (etching, deposition and treatment, see below) processes.

The depth of the plasma-activated surface modification processes can generally be designed with great accuracy within 10^1–10^4 nm; this certainly qualifies plasmas as nanotechnologies for the vertical direction. For the lateral directions, indeed, PST processes can be optimized to the homogeneous transfer of the modifications on very large area substrates of industrial interest, batch (e.g., glass panels for solar cells) or in continuous (or semicontinuous) motion at high rate (e.g., polymer webs for food packaging). Three kind of surface modification plasma processes can be defined in general, as it is described in the next: ablation, plasma deposition, and plasma treatment,

Ablation (dry etching). Ablation reactions, namely, the formation of volatile compounds after reactions between active species in the plasma and substrates (e.g., halogen atoms vs. silicon, oxygen atoms vs. polymers and other carbon-based materials, etc.), can be precisely designed also with lateral resolution of tens of nanometers and very high aspect ratio, by coupling PST with micro-/nano-photolithographic techniques. The word etching is used, rather than ablation, to define this way of 3D "sculpting" silicon and other materials at nanometric scale. Dry (plasma) etching processes, still in evolution to satisfy the needs of extreme miniaturization and newer materials,[6,9] have overridden wet etching methods in the 1970s, and are at the base of the extreme and continuous miniaturization of processors in Microelectronics. Deep plasma etching "micromachining" processes are also available in PST for "drilling" holes deep tens of micron through silicon wafer or other materials for advanced microfabrication procedures.[6] Also, certain etching processes can be optimized to the production of rough "textured" surfaces characterized by micro/nanometric reliefs for developing superhydrophobic or superhydrophilic surface properties.[10] References[1,6] describe the evolution of plasma etching technologies since the beginning till today.

Plasma Deposition. Also known as plasma enhanced chemical vapor deposition (PE-CVD), depending on the LP/AP experimental plasma conditions and on the nature of the feed, it allows decoration of substrates with coatings of many possible chemical compositions, cross-linking and properties. Table 2 show a nonexhaustive list of classes of PE-CVD coatings, of their application, and of feeds they can be deposited from in different experimental conditions.

Also compounds such as saturated hydrocarbons, fluorocarbons and organosilicons, unsuitable for conventional polymerization reactions for the lack of proper chemical functionalities (e.g., carbon–carbon double bonds) can deposit PE-CVD coatings. This is a peculiar feature of PE-CVD processes with respect to other technologies.

The building blocks of PE-CVD coatings, whose thickness is generally kept within 5–10^3 nm, are radicals (mostly) and other neutral species generated in the plasma by the fragmentation of the feed. Since many possible fragments originate in the same process, the stoichiometry of the coatings can be varied in continuo with the discharge parameters, very differently from conventional polymers. In spite of this, however, when organic compounds are used as feed, the terms "monomer" for the compound, "Plasma Polymerization" for the technology, and "Plasma Polymers" for the coatings are utilized in the PST jargon.

Table 2 PE-CVD coatings deposited in LP/AP cold plasmas

Coating, composition	*Feed*	*Applications*
Teflon-like, CFx	Fluorocarbon-based: C_2F_4, C_2F_6/H_2, C_3F_6, etc.	Hydrophobic and super-hydrophobic surfaces
Silicone-like, SiCxHyOk	Organosilicon: hexamethyldisiloxane, HMDSO; tetraetoxysilane, TEOS; etc.	Hydrophobic and super-hydrophobic surfaces
Silica-like, SiOx	Organosilicon/O_2: HMDSO/O_2; TEOS/O_2; etc.	Gas/vapor barrier layers in food packaging; anti-corrosion coatings on metals; anti-tarnish coatings on silver and alloys; anti-scratch coatings on plastic optics; dielectric layers in microelectronics
Hydrogenated amorphus silicon (a-Si:H)	Silane-based: SiH_4/H_2; etc.	Photovoltaic coatings for solar cells
Diamond	Hydrocarbon-based: CH_4/H_2; etc.	Hard coatings for cutting tools; IR-transparent windows
Diamond-like carbon (DLC)	Hydrocarbon-based: CH_4/H_2; etc.	Anti-wear coatings; gas/vapor barrier coatings in food packaging; blood-compatible coatings
PolyEthyleneOxide(PEO)-like	Glycols: $CH_3O(CH_2CH_2O)_nCH_3$, $n = 2$–4, ⋯	Coatings nonfouling in water media.

Hardness, hydrophilicity and hydrophobicity, protection against oxidation and corrosion, barrier against gas/vapor diffusion, lubricity, wear resistance, resistance to bacterial colonization, cyto-compatibility and many other properties can be imparted at the surface of substrates by means of PE-CVD. One of the advantage of this technology is the ability to "nobilitate" conventional substrates with the application of a submicron thick layer characterized by predetermined composition and properties, absent in the original substrate.

To some extent, PE-CVD is related to other thin film deposition techniques, namely, chemical vapor deposition (CVD) and physical vapor deposition (PVD, evaporation, sputtering). In CVD the building block of the coating (e.g., carbon) are formed by the pyrolysis of a feed gas at high temperature; in sputtering PVD processes atoms and clusters precursors of the coating (metals, oxides, nitrides, etc.) are ejected by a cathode (target) electrode hit by energetic positive ions of an inert gas generated by a LP plasma. It is possible to couple PE-CVD and sputtering in the same reactor, and deposit nano-composite coatings consisting, for example, of nanometric clusters of a metal or of an oxide embedded in a PE-CVD matrix. Several combinations of PE-CVD/sputtering processes can be arranged; this has generated classes of nano-composite coatings with many possible applications, from hard coatings to antibacterial layers.[11,12]

In a recent evolution of PE-CVD technology, AP-DBD processes fed with the water aerosol of biomolecules (proteins, peptides, enzymes, and so on) have been investigated,[13] capable of depositing bio/nano-composite coatings with biomolecules embedded in a polymer-like matrix, releasable in water media in active form.

Plasma Treatments. In Plasma Treatments materials are exposed to LP/AP cold plasmas fed with reactive (e.g., O_2, N_2, H_2, NH_3, H_2O vapor, N_2/H_2, etc.) or inert (Ar, He, etc.) nonpolymerizable feeds. The surface modifications imparted in this way are extremely shallow, few nm, and include cleaning from surface contaminants, oxidation, cross-linking and grafting of chemical groups at the surface of polymers. Grafting oxygen/nitrogen containing polar groups onto polymers, for example, allows to impart hydrophilic character, printability, affinity with other materials (e.g., better fiber/matrix or particle/matrix adhesion in composite materials),[14] cyto-compatibility, as well as "anchor groups" for the immobilization of biomolecules in subsequent conventional wet processes. Grafting F atoms, instead, lead to surfaces with increased hydrophobicity. Generally, the recovery of the substrate polymer chains has to be controlled as a function of the surrounding environment (e.g., by previously cross-linking the uppermost layers of the substrate), in order to stabilize the treated surface.[15–17]

Plasma Reactors and Plasma Sources

Several architectures are available of plasma reactors and sources; the choice of the source, of its features and performances highly depends on the application and on the LP/AP pressure regime of the selected process. The parallel-plate reactor has to be cited, sketched in Fig. 1 in its simplest configuration, among the most popular design of LP plasma reactors for material surface processing. RF power supplies at 13.56 MHz are often used for this reactor configuration. Parallel-plate reactors are among the most utilized in PST of semiconductor and in the IC production in Microelectronics, as well as in other fields. They can be designed in many possible configurations and engineered at many possible level of sophistication, depending on the substrate (large, small objects, micro-/nano-particles, batch, in movement, etc.), on the throughput (lab scale, industrial) and on the selected application. For processing substrates in form of webs (polymers, textiles), for example, the reactor is implemented with winding/unwinding roll-to-roll equipment.

LP plasma reactors often are properly configured in a way (e.g., three electrodes "triode" configurations, superimposed magnetic field, etc.) that allows to finely control and drive the energy of the vertical bombardment of positive-ions impinging on the substrates independently on the chemical action of neutral etchant species with the substrate. This feature is of top importance in

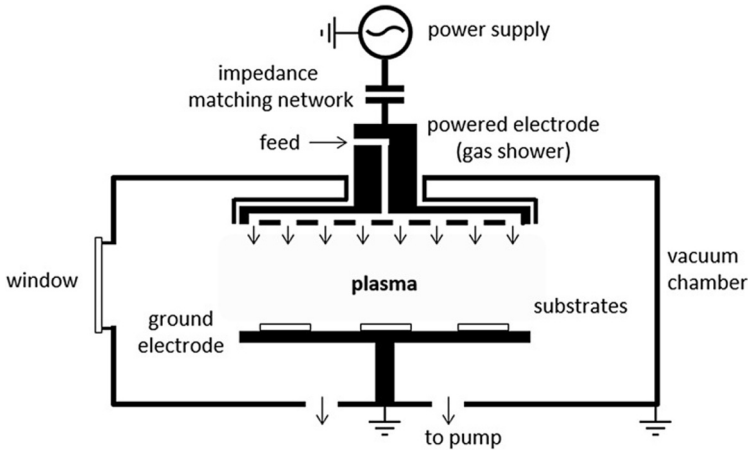

Fig. 1 General sketch of a LP parallel plate plasma reactor.

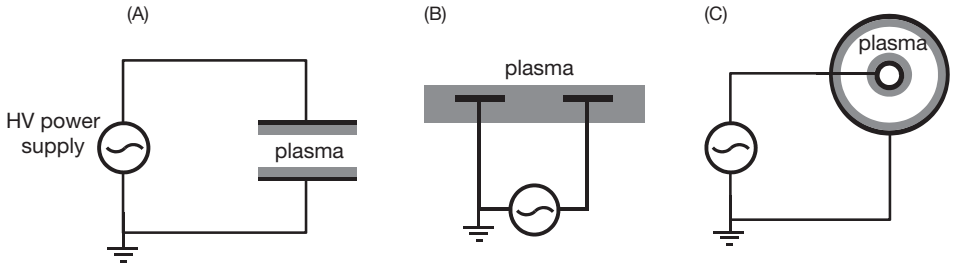

Fig. 2 Design of the three possible configurations of AP-DBD: (A) parallel-plate DBD; (B) coplanar surface DBD; (C) coaxial DBD. The dielectric layer that covers the electrodes is represented in *gray*.

Microelectronics, since it allows to enhance the vertical etch rate with respect to the lateral one, thus obtaining high resolution anisotropic etching of materials at nanometric scale with very high aspect ratio.[6,9]

Along with corona discharges, dielectric barrier discharges are today among the most widely used experimental set-up to generate cold AP plasmas. DBDs usually generate filamentary AP plasmas that may result in nonhomogeneous modification processes at the surface of materials; by properly optimizing the experimental set up, however, it is possible to achieve homogeneous DBD-AP processes.[8,16] In DBDs a layer of dielectric material (alumina, quartz, etc.) is used to cover one or both electrodes, in order to reduce the current and generate AP plasmas close to or at RT. Electrodes are usually separated by a gap of a few millimeters, due to the higher breakdown voltage of gases at AP; this feature may be seen as a drawback with respect to LP processes, were much larger electrode gaps are common, feasible for processing most kind of substrates. DBD electrodes are connected to an AC generator, typically in the KHz range, driven at several kV of voltage.[7,8,18,19] The AC field is often applied in pulsed regime, with fast time-rise pulses of micro/nanosecond width,[7,19–21] to prevent the transition from a glow (cold) to an arc (thermal) discharge. The same approach allows to obtain homogeneous rather than filamentary discharges, and to keep the gas temperature very stable at RT. This condition, in particular, has to be fulfilled in certain medical therapeutic applications of AP discharges (e.g., in wound healing), where the temperature of the discharge effluents has to be kept below the temperature of the living tissues they come in contact with.

With respect to LP regime, AP discharges are usually fed with higher gas flow rates (liters per minute vs. tens/hundreds mL per second). Quite often, a buffer noble gas (He, Ar) with lower amounts of other volatile compounds is utilized as feed. In spite of the fact that He is very expensive, still it is regularly used in academic AP plasma processing research due to its low breakdown voltage (about 4 KV/cm), and to its ability to generate more easily diffuse stable discharges with respect to other gases.[19] The design of three common DBD configurations is sketched in Fig. 2.

Cold AP sources can be also properly designed to produce plasma plumes or afterglow effluents investing the substrate at some distance (millimeters–centimeters) from the source itself. Originally developed as thermal plasma torches for propulsion and for high T cutting and welding of materials, these plasma sources are often referred to as AP Plasma Jets (APPJ).[20,22,23] Among other possible configurations, the DBD architecture can be conveniently used also for APPJs. Two of the many possible APPJ designs are shown in Fig. 3.

APPJ sources are being used to further develop several established and emerging PST applications, such as surface engineering, micro-/nano-materials synthesis, and medical protocols. Many APPJ configurations for surface engineering applications are described in.[24]

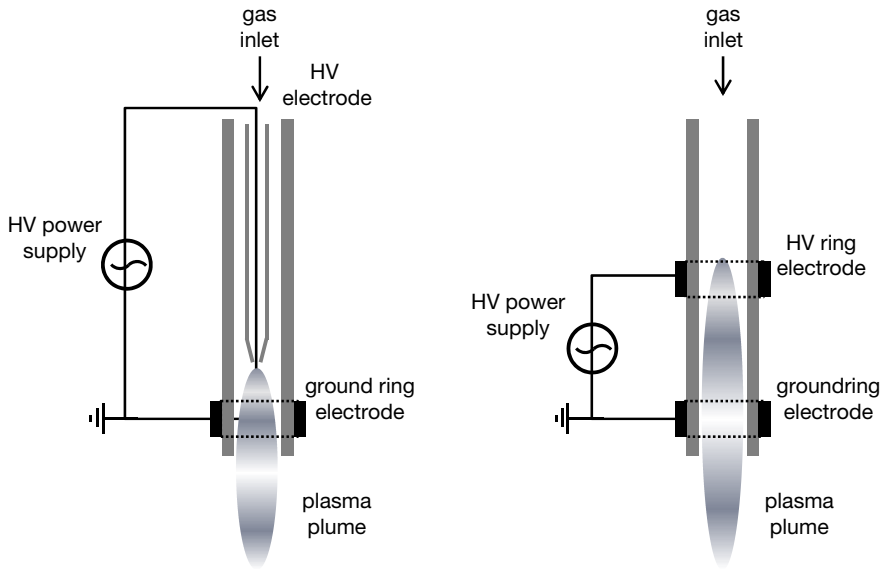

Fig. 3 Design of two APPJ plasma sources. Glass (dielectric) tubes are in *gray*.

Plasma Diagnostics, Surface Analysis, and Process Control in Plasma Science and Technology

PST has advanced enormously in time; the ability of scientists and technologists in predicting and planning the performances of plasma processes has developed toward better understanding and newer applications, thanks to the extensive use of plasma and surface diagnostic tools as well as of computational skills. A proper use and correlation of diagnostic techniques of the plasma phase and of the processed surfaces is of fundamental importance for understanding the chemical mechanisms of production of the active species in plasmas, and for mastering the effects of the experimental parameters on the plasma processes, in order to drive and optimize them toward preselected surface modification channels. This is clearly important for the academia, but is essential for the scale-up of the processes in the many industrial fields where PST is utilized.

For the plasma phase, optical techniques (optical emission spectroscopy, OES; laser induced fluorescence, LIF; absorption spectroscopy, AS; etc.) are very much utilized for their non-invasive character; mass spectrometry (MS) and langmuir electrostatic probes (LEP; etc.) are also utilized, although to a lesser extent. Coupling plasma diagnostics with surface chemical/morphological analysis techniques is mandatory to properly understand, predict and scale up the effect of LP/AP plasmas on surfaces. Electron spectroscopy for chemical analysis (ESCA), contact angle (CA), scanning electron microscopy (SEM), atomic force microscopy (AFM) and secondary ion mass spectrometry (SIMS) are just the most popular surface techniques used in this field, among many others. Any investigation finalized to the best understanding of a particular surface modification plasma process has generally to be completed with finding correlations between the chemical composition and the morphology of the processed surface, and the resulting properties of interest for the selected application. References [24,25] by Favia et al. show examples of correlations between OES and ESCA data for PE-CVD and plasma treated polymers of biomedical interest.

Plasma Technology for Life Sciences

One of the first experiments dealing with PST and Life Sciences has been described in 1953 by S.L. Miller,[26] who applied corona sparks for some days in a row to a gas/vapor mixture of methane, ammonia, hydrogen and water vapor, the plausible components of primordial atmosphere on Earth, to imitate the effects of lightning. Amino acids, usually found in proteins, could be detected in the sealed glass vessel after the experiment, and a consistent hypothesis on how Life started on our planet was confirmed.

Plasma science and technology nowadays impacts three different large areas of medicine and biology, as defined below.

Surface Engineering of Biomedical Materials. Biomaterials are nonviable materials used in medical devices (catheters, plastic wares, all kind of prostheses, scaffolds for regenerative medicine, biosensors, sutures, etc.), intended to interact with biological systems in vitro, ex vivo and in vivo.[27] In this particular field, etching, deposition and grafting plasma processes are developed at the surface of biomaterials and biomedical devices to properly functionalize it and elicit the optimal response of biological entities (proteins, cells, bacteria, fluids, tissues) in contact with them, as described in.[28] Chronologically, this is the first and still the wider area of applications of cold plasmas in the biomedical field.

Sterilization and Decontamination of Materials and Devices. The surface decontamination of biomedical materials from biological and organic molecules, as well as their sterilization from bacteria, is one of the several applications of plasmas that have

been boosted by the diffusion of AP plasma discharges in the early 1990s, almost at the same time when LP plasma-sterilizers for hospitals became available in the market. Plasma decontamination/sterilization of materials is due to the lethal effects of reactive species (e.g., O atoms, OH radicals, etc.) and UV photons generated in plasmas on bacteria and spores.[29] This wide and strategic application area of PST is being nowadays extended also to food stuff, seeds, plants and liquids.[30]

Plasma Medicine. The term Plasma Medicine defines the emerging field of application of cold plasmas, where living tissues are directly exposed to AP cold plasmas in air, for eliciting therapeutic effects such as wound sterilization and healing, tissue regeneration, blood clotting and killing of cancer cells. AP plasmas are also being experimented for sterilization of dental cavities, teeth bleaching, treatments of acne, and other therapies. This recently born discipline combines plasma physics, plasma chemistry, life sciences and clinical medicine in what is probably today the most promising field of applications of PST.[31,32]

Other areas of applications of plasma science and technology sprouted recently from plasma sterilization and plasma medicine, where potential uses of cold plasmas are investigated in Agriculture, Food and related fields for improving the germination of seeds and for the decontamination of fruits, vegetables, and other food products.[30]

The next section are dedicated to describing biological tests to assess cell response to (plasma-modified) surfaces.

Biological Investigations on Biomaterial Surfaces

Examples of nonbiological materials used into the human body are known in history, from nacre teeth made from sea shells by the Mayans and linen sutures used by the early Egyptians, to modern pacemakers and prostheses.[33] The discipline of Biomaterials Science is advancing rapidly and continuously,[33] impacts human health, ethics and economy, and takes advantage of knowledge stemming from other fields like Material Science, Engineering, Chemistry Biology and Medicine, also including Ethics and Law.

Metals, ceramics, polymers, and composite materials are commonly utilized in advanced prosthesis and medical devices, and hybrid assemblies of biodegradable polymers and cells are engineered to be implanted as tissue substitutes and develop similarly to the host tissue after the degradation of the polymer, in the disciplines of Tissue Engineering (TE) and regenerative medicine.[34] The surface of the material is the main site of the interaction between any biomaterial and the surrounding biological media (cells, bacteria, blood, fluids, tissues, etc.), in vitro and in vivo; the way this interaction, developed in the host biological system, ultimately determines the success or the failure of the biomaterial in its function. In this contest, all surface modification techniques are clearly of interest for tuning the surface properties of biomaterials, with the aim of producing an appropriate response from cells and tissues (e.g., firm and durable integration of the bone tissue of the jaw with the titanium surface of a dental implant) while they interact with the material at its surface.

Testing properly the "biocompatibility" is of vital importance for the optimization and validation of biomedical devices, where biocompatibility is defined as "the ability of a material to perform with an appropriate host response in a specific application," in reference to the interactions between materials and biological systems.[32,33] Examples of "host responses" are: blood compatibility and absence of blood clotting (e.g., for artificial heart valves, vascular prostheses, and hemodialysis systems); osteo-integration (e.g., between bone tissues and orthopedics prostheses or dental implants); antibacterial properties, and so on. Host response clearly depends also on the in vitro (e.g., cells in polystyrene culture dishes), ex vivo (e.g., extra corporeal blood circuits) or in vivo (e.g., implants and prostheses) nature of the interaction, as well as on its duration, that can span from hours (catheters, hemodialysis membranes, etc.) in the case of disposable devices, to lifetime (pacemakers, hip joint prostheses, etc.) for prostheses.

As for other surface modification technologies, PST is often utilized for driving the interactions between the biological host (bacteria, cells, tissues, fluids) and the surface of a biomaterial, so it is of crucial importance to compare the biological response to modified materials with respect to unmodified ones, to properly check whether their biocompatibility was improved.

When a biomaterial is placed in contact with a tissue of the body, a number of biological processes occur at the surface of the material, such as protein adsorption and desorption, macrophage activation and production of foreign body giant cells, and tissue/organ specific cell responses (e.g., proliferation of endothelial cells, immune cell response, tumor formation).[35]

In order to verify if cells react properly to a material, as it or surface-modified, in a biological environment, with no harmful reactions, in vitro biocompatibility tests are utilized. If the material is found nontoxic, in vivo clinical tests follow, generally consisting in implanting the material in an animal model and in the evaluation of its histocompatibility. Clinical trials in human patients may follow after animal tests. Both animal and human trials require mandatorily the previous approval from the institutional ethical committees.[36]

In vitro cell culture assays allow easily and reproducibly to attest the cytocompatibility of material surfaces to tissues and cells.[37,38] Using single cell types is very convenient to investigate in-depth specific cell–surface interactions, a study not possible in vivo, where many different effects would take place.

The selection of cell types should be based on the specific application under investigation. For instance, Schwann cells and neuroblastoma cell lines can be used to assess the in vitro cytotoxicity of materials used for nerve regeneration,[39] human fetal osteoblast or osteosarcoma cell lines can be used to check the cytocompatibility of orthopedic implant materials,[40,41] keratinocytes or fibroblasts, instead, for determining the cytotoxic potential of wound dressing materials.[42]

In vitro essays mainly use primary or immortal cells. Primary cells are obtained directly from the mechanical or enzymatic disaggregation of a living tissue; their behavior is close to that displayed in vivo, though with a short life span, and slow proliferation.

Primary cells isolated from different donors can behave differently in culture conditions depending on the genetics and on the age of the individual from whom the tissue was taken, and same type of cells, for example, fibroblasts, that derived from different parts of the body may have different characteristics. Cell lines (consisting of immortalized cells), instead, are easy to use in biological studies, for their uniformity of cell population, for their high proliferation rate, and for their resistance to long-term storage. For these reasons the scientific community tends to accept scientific data more readily when they are based on cell lines experiments, since these have been considered the standard for similar research in the past, usually showing high reproducibility of results. However, despite the aforesaid clear advantages since cell lines do not represent exactly what actually occurs in vivo, it is a good practice to perform the same experiments also with primary cells to possibly replicate the results with cells showing a similar behavior to in vivo.

Cytotoxicity tests use tissue cells in vitro to evaluate the toxicity of materials due to released compounds and/or to other effects. They are generally the first to be performed in the evaluation of medical materials and devices.[43] Using several different biocompatibility tests might generate more biologically relevant information concerning the nature of the toxicity.[44] For example, high concentrations of reactive oxygen species (ROS, see "Sterilization and Decontamination of Materials and Devices" and "Plasma Medicine" sections) inside cells can be monitored by a fluorescent product (DCF assay) generated by the oxidation of the nonfluorescent compound, H2DCF-DA. Likewise, using colorimetric assays based on the reduction of light absorbing substrates, such as MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and LDH (lactate dehydrogenase), it is possible to investigate the cellular metabolic activity and the cell death, respectively.[45]

Cell morphology, adhesion and spreading provide clear indications about the cellular responses to external stimuli such as plasma treatments.[46] This task is generally accomplished by fixing the cells atto the substrate after different time of growth, followed by staining with dyes for easier observation and measurement of their density at the optical microscope. More specific microscopic techniques, including fluorescence microscopy, confocal microscopy, and scanning/transmission electron microscopy, are generally employed to study cell-substrate interactions and other specific cells features. The cytoskeletal organization of the cells is commonly determined after staining actin with fluorophore-labeled phalloidin: this approach is normally used to assess cell motility, cell spreading, and cell shape.[47] Moreover, confocal laser scanning microscopy has the advantage to probe the cell–material interactions by means of the simultaneous 3D visualization of both cell morphology and support architecture.[48] Due to its high resolution, SEM is one of the most frequently used imaging tools to get an overview of the cell distribution and surface coverage on biomaterials. Even though transmission electron microscopy (TEM) offers the highest possible magnification and resolution, a time consuming series of sample-processing steps and the requirement of ultra-thin sectioned samples, make it a less commonly utilized tool for evaluating cell–material interactions.

Surface Engineering of Biomedical Materials

The ability of modifying the surface of materials with no alterations of the bulk, in a sterile medium, became soon obviously of interest also in biomaterials science, a field that has experienced an impressive interdisciplinary growth in the last decades, with strong economic impact.[33] The first papers dealing with surface modification plasma processes of biomedical interest, in particular, were published in the late 1960s; approximately in the same period the application of cold plasmas started to be investigated in Microelectronics and Semiconductors. Nowadays a large arsenal of deposition, treatment, and etching LP/AP plasma processes is available in the academic literature for developing surface modification protocols of biomedical materials. The scope of this approach is generally that of tuning the surface properties of biomedical materials to the best predetermined interactions with biological entities. Many of these processes actually have led to products available in the market. Some of them are part of more complex "surface decoration" protocols of functionalization with biomolecules (e.g., peptides, polysaccharides, etc.), aiming at imitating the 3D biochemical nature of biological entities (e.g., the extra cellular matrix) on top of conventional materials, with the scope of facilitating their cell colonization and tissue integration in the most similar possible way to what actually happens in vivo.

The next sections list surfaces of biomedical interest that can be developed with the aid of LP/AP plasma processes.

Biomimetic Surfaces

Cell adhesive

Plasmas are often used to insert chemically reactive functionality onto otherwise nonreactive substrates. Several reviews report on plasma polymerization and treatment of biomaterials,[49–53] but here we will review a select set of plasmas, those that lead to surface chemistries suitable for promoting cell-adhesion. It is well established that cell adhesion to an artificial material strongly depends on the physico-chemical properties of the material surface. For example, the presence of oxygen-containing functional groups increases energy, polarity, and wettability of the material surface, and supports the adhesion and growth of cells.[54,55] Moreover, oxygen-containing groups increase the adsorption of adhesion-mediating ECM proteins, for example, vitronectin, fibronectin, collagen or laminin. At the same time, the adsorption of cell repulsive molecules, for example, albumin, is attenuated because these molecules prefer to bind to less oxygenated and more hydrophobic surfaces.[56] Material surfaces plasma functionalized with carboxylic groups have been found to be good supports for some anchorage-dependent cell lines.[57,58] On functionalized self-assembled monolayers, the amount of adsorbed fibronectin decreased in the following order of surface functionalities: $NH_2 > CH_3 > COOH > OH$, while the adhesion of MC3T3-E1 osteoblast-like cells, mediated by $\alpha 5\beta 1$ integrin adhesion receptors,

increased in a similar order, that is, $CH_3 < NH_2 = COOH < OH$, which can be explained by changes in the geometrical conformation of fibronectin.[59] Amino containing plasma functionalized surfaces can be produced both with plasma grafting from nonpolymerizable gases (i.e., NH_3, N_2/H_2, N_2, etc.) and with plasma polymerization of N-containing monomers (i.e., allylamine). The density of amines created by plasma polymerization of allylamine were reported to be higher than for NH_3 or N_2/H_2 plasma-treated substrates.[60]

Nano-textured

Scientists have learned from nature to control some unique properties such as extreme wettability, antireflectivity, antifog, and color shading, by manipulating surface micro- and nano-texture. It is, in fact, well known that sculpting submicro texture onto a surface can abruptly change wetting and optical properties of a material as illustrated in Fig. 4. Materials presenting such properties can find application in a variety of fields, from clothing to optics and from low friction navigation to tissue engineering.[61–66] The latter application is based on the capability of cells to sense the topography and chemistry of the surface to which they adhere on.

Many methods have been developed to produce nanoscale controlled topography onto polymer surfaces, including colloidal lithography,[67] microcontact printing,[68] electro-spinning.[69] These approaches are somehow complex and expensive, and often the main concern lies on the possibility to exploit the process on large area.

A simple method for preparing nano-textured surfaces, scalable to large area, is *plasma texturing* (or plasma roughening): often in a matter of minutes, random nano/submicrometric features can be prepared onto polymeric substrates, depending on plasma conditions.[63,65,70–72] The process is based on plasma etching through a self-masking mechanism: due to the controlled surface contamination by metal or oxide clusters, a random nano-featured mask is formed onto the surface and etchant species can attack only through the open regions.[73] This method allowed for the preparation of superhydrophobic surfaces onto PMMA[71] and PS,[63,66] antifog surfaces onto PC[65] and of antireflective silicon.[73,74]

Controlling plasma etching and deposition to combine optimum surface topography and chemistry can be useful to tailor polymer surfaces in order to tune cell adhesion and proliferation. Despite the plasma approach is quite easy, only few works in literature concerns direct plasma roughening of polymeric materials for controlling the biological response.

Tserepi et al. nano-textured PMMA via direct oxygen plasma etching, tuning the surface morphology by applying different bias voltage at the substrate, with a short process duration: 1 min.[70] They have found that the adhesion of 3T3 fibroblast dramatically increased on treated PMMA surface. However, it should be considered that upon plasma texturing also the surface chemistry was changed, since carboxylic acid groups were formed, and the effect of chemical composition can be difficult to disentangle from the morphology one in such experiments. Nevertheless, the same authors exploited a similar approach to restrict cell growth in topographic domains to produce cell arrays.[71] In particular, as depicted in Fig. 5, combining photolithography and oxygen plasma roughening of PMMA and subsequent selective deposition of a hydrophobic Teflon-like coating, they could obtain microchannels alternating superhydrophobic and superhydrophylic regions. HT1080 cells, cultivated on such modified microchannels, adhered only on the superhydrophilic areas of the microchannel and were completely repelled from the superhydrophobic ones.

Muhymin Islam et al. demonstrated that tunable nano-texturing of PDMS can be carried out by means of a CF_4/O_2 plasma with consequent effects on cells growth and proliferation.[72] Fibroblasts, hGBM and human astrocyte cells were seeded onto plasma treated PDMS surfaces modified with aptamer molecules responsible for cell attachment and capture. It was found that nano-texture effectively enhanced the growth rate of cultured cells compared to plain surfaces.

Biomolecule immobilization

Materials commonly found in the biomedical field are polymers like polyethylene (PE), polyethyleneterephtalate (PET), polystyrene (PS) or polytetrafluoroethylene (PTFE), but also inorganic ones such as silica or metals. These materials are often reluctant to functionalization, necessary to form on the surface binding sites for active molecules, if not in harsh conditions. Hence, different kind of plasma treatment or PE-CVD deposition can be advantageously used to overcome this issue.

The general approach is schematized in Fig. 6: two steps are commonly followed. During the first step, the material is exposed to plasma for addition of the chosen functional moieties to the surface. These can be N-containing groups, typically amine,[75] or O-containing ones (—OH, —COOH, epoxy)[76,77] and they can be directly grafted on the surface or brought with a plasma deposited coating. Then the functionalized material is dipped in a solution for coupling the biomolecule. However, two different routes can follow: the solution can simply contain the biomolecule (beyond additives), that, then, is adsorbed more or less firmly onto the surface. On the other hand, covalent binding is more desirable for permanent bio-functionalization and many wet chemical

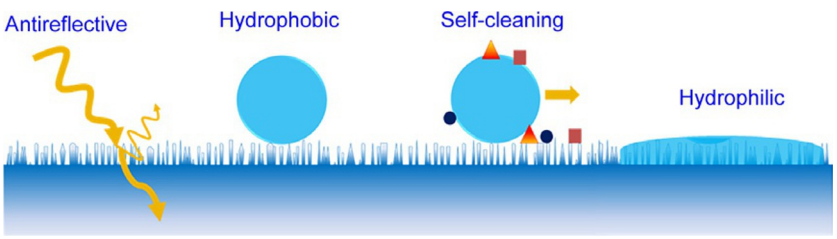

Fig. 4 Material properties influenced by surface micro-, nano-texturing.

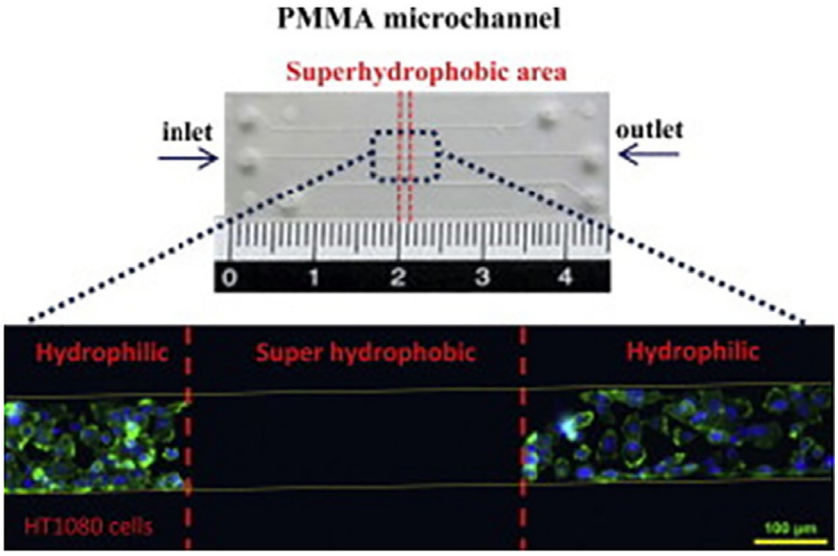

Fig 5 Fluorescence image of HT1080 cells cultivated on a microchannel with variable wetting characteristics (superhydrophilic, contact angle CA $<10°$, superhydrophobic, CA $\sim 150°$) formed in PMMA by O_2 plasma etching and deposition of a hydrophobic Teflon-like film in the middle zone. Reprinted from Copyright 2014, with permission from Elsevier Ltd. Tsougeni, K.; Bourkoul, A.; Petrou, P.; Tserepi, A.; Kakabakos, S.E.; Gogolides, E., *Microelectron. Eng.* **2014**, *124*, *25*, 47–52.

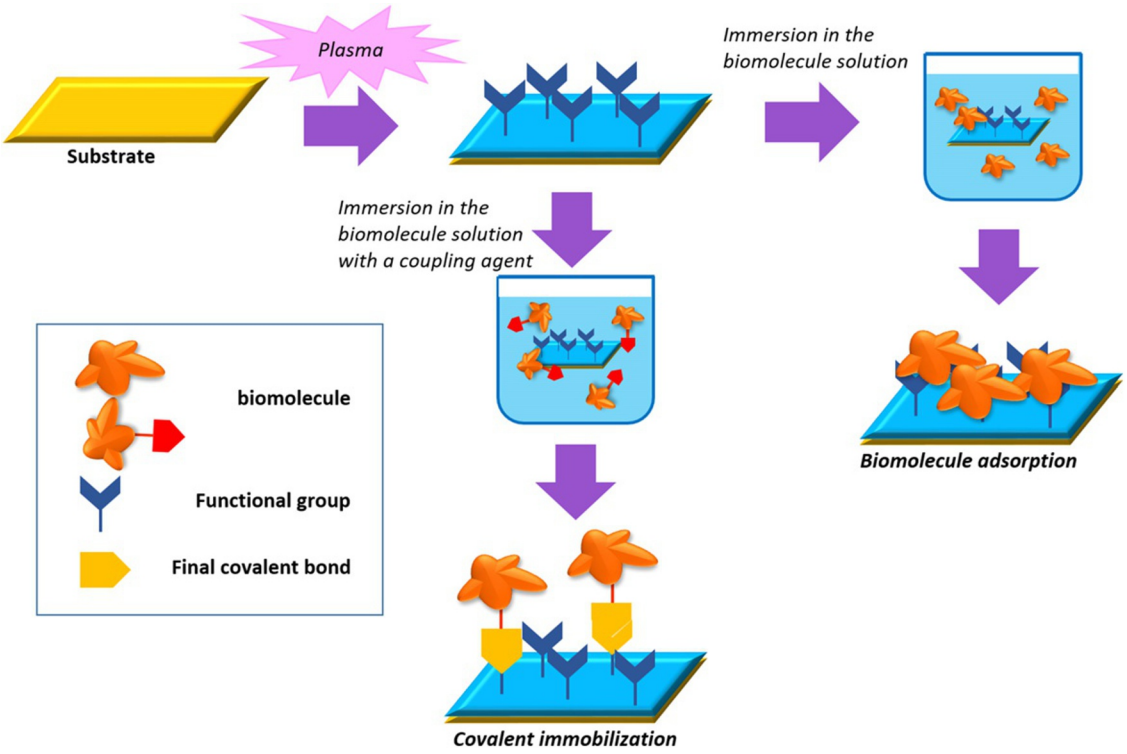

Fig. 6 Approach to biomolecule immobilization through plasma functionalization.

methods have been reported in literature for this purpose.[78–81] Carboxyl (COOH) groups on the surface can be activated to form amide groups through the carbodiimide chemistry, using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, water soluble) and alike, to react with amines side chains of proteins or other biomolecules. This approach can also be carried out to link carboxyl groups of the bioactive compound to amino groups grafted onto surfaces. The latter method deserves some precaution since when immobilizing peptides or proteins (and molecules bearing both acid and amino group) undesired crosslinking could result.[77] In

some cases linking of spacer molecules between the surface and the biomolecule has been reported in order to limit denaturation or steric hindrance of the bioactive agent. Typical spacer arms are succinic anhydride bis-amino-poly(ethylene glycol), or similar bis-amine functionalized molecules.[82]

The main reason for grafting biomolecules on functionalized biomaterial surfaces is to mimic the surface composition of a tissue or organ or its parts, in order to help the integration of biomaterials into the human body. Several attempts are reported, for instance, to graft the RGD oligopeptide onto —COOH plasma-deposited functionalized coatings. The RGD (Arginine—Glycine—Aspartic Acid) amino-acid sequence is the most frequently used peptide to stimulate cell adhesion on synthetic surfaces, since it represents the minimal adhesion domain of most extra-cellular matrix proteins (e.g., fibronectin, vitronectin, collagen, etc.) and it is able to specifically promote cell adhesion, spreading and growth.[83]

Some authors used mild experimental conditions and proper flow rate ratios of ethylene and acrylic acid to deposit stable coatings on different substrates functionalized with carboxylic groups. Using EDC activation, —COOH groups were covalently bonded to the- NH_2 side of bis-amino terminated PEG (polyethyleneglycole) spacer arm, connected itself with the C-terminus of the RGD tripeptide in a fast reaction taking place in aqueous environment. This strategy allowed the enhancement of endothelial cells growth on PTFE surfaces,[75] supporting the long-term maintenance and differentiation of human liver cells on PES membranes for bioreactors,[84] and drove the adhesion, spread and proliferation of fibroblast cells on PET substrates.[85]

The conjugation of biomolecules to plasma-functionalized surface could locally deliver a precise message to the target cells, giving them inputs for metabolic changes. This is the case of molecular signals like growth factors, when they are used to induce cell proliferation/differentiation on the surface of biomaterials. Puleo et al. used carbodiimide-mediated coupling reactions to attach the Bone Morphogenic Protein-4 to titanium coated with amine-rich plasma-coatings deposited from allylamine. This strategy allowed the improvement of bone regeneration on titanium surfaces, a material of election for the manufacturing of bone prostheses.[86]

Vascular endothelial growth factors (VEGF) are important for vascularization in tissue engineering applications, to regulate cell signalling, migration, proliferation, and differentiation of endothelial cells.[87] Nano-fibers of poly(ϵ-caprolactone) (PCL) were produced by electrospinning and coated in a radio frequency plasma process fed with ethylene/CO_2/Ar mixture to get carboxylic acid groups rich surfaces. VEGF was covalently immobilized to the scaffold using hexamethylenediamine as a linker. ELISA immunoassay demonstrated a surface growth factor concentration between 4.5 and 33.3 ng/mm^2, furthermore the biological activity of immobilized VEGF was maintained and, in particular, bio-functionalized scaffolds induced a higher cell proliferation after 9 days culture with respect to untreated scaffolds.

Non Fouling Surfaces

Fouling is a main concern in the case of surfaces exposed to aqueous environment where a conditioning layer composed by protein and/or cells can be formed, providing an easily accessible platform for other undesired species to attach and proliferate. The adhesion of such promoting layer would be lower onto hydrophobic/superhydrophobic surfaces since driven mainly by weak forces (e.g., Van der Waals).[64] Hydrophilic polyethylene glycol (PEG) polymers, also referred to as polyethylene oxide (PEO), have been shown to resist proteins and cells (including bacteria) attachment.[88] Such surfaces are defined as “nonfouling” or “antifouling”.[89,90] This property is believed to strongly correlate with the hydration layer at the PEO surface,[45] due to the hydrophilic ether $(CH_2—CH_2—O)_n$ functionalities. These groups create a water-solvated structure, which forms a liquid-like surface with highly mobile disordered molecular chains.[91] For protein adsorption to occur, there must be a reduction in the dehydration entropic energy associated with the removal of surface bound water.[92] Due to this effect, the tightly bound water molecules entrapped in the PEO surface through hydrogen bonds form a physical and energetic barrier that cannot be displaced by proteins and cells. LP PE-CVD processes from monomers bearing CH_2CH_2O moieties have been widely applied as a versatile tool to impart nonfouling properties with PEO-like coatings on a large variety of substrates.[93–97] Three important features have to be achieved for these coatings: good adhesion to substrates, stability in water media, and high retention of the CH_2CH_2O functionalities of the monomer. This latter parameter is often referred to as “PEO character,” and it can be evaluated by measuring the relative importance of the ether carbon component at ~286.5 eV of Binding Energy in the C1s XPS spectrum of the coating.[98] High PEO character is desirable in nonfouling surfaces for proteins and cells; coatings with low PEO character, instead, promote protein and cell/bacteria adhesion and proliferation.[99] Beside LP approaches, AP plasma assisted ones have also received, recently, great attention for the deposition of PEO-like coatings.[90,100]

Antibacterial, Antiviral, and Antifungal Surfaces

Plasma processing for preventing microbial colonization of biomaterials is considered a valid strategy, often, not just at the level of implant/tissue interface but even deep in the surrounding tissues. One of the major issues in this field is represented by device-related infections (DRIs) due to bacterial colonization and proliferation of indwelling devices. These infections generally require a long period of antibiotic therapy and occasionally repeated surgical procedures, resulting in potential risks for the patient and increased costs for the healthcare system. A promising alternative to systemic therapies is the design of devices with surface properties aimed at locally modulating interfacial interactions between implanted devices and host tissues by preventing bacteria attachment and proliferation. Bacterial colonization of medical devices leading to DRIs is, in fact, a surface-mediated process, and poses challenging problems to biomaterial scientists.

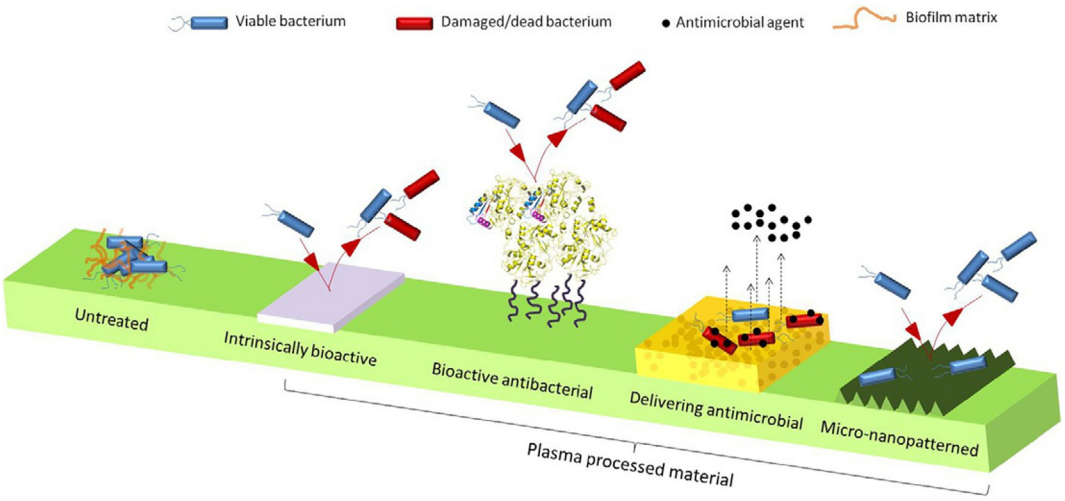

Fig. 7 A glance to engineering antibacterial surfaces using plasma-based tools in order to contrast infections of medical devices.

Surface characteristics ranging from chemistry to roughness, can be properly tailored by plasma assisted approaches in order to control bacterial adhesion and growth. Different surface modification approaches aimed at producing antibacterial surfaces can be considered as summarized in Fig. 7: intrinsically antibacterial surfaces; bioactive antibacterial materials; biomaterials delivering antimicrobials; micro and nano-structured materials.[101–104]

Intrinsically antibacterial surfaces

Biofilms are extremely hard to remove and show great resistance to all kinds of biocides, thus, their prevention is an important step to avoid spreading of diseases and material deterioration.[105] To limit the biofilm formation on a surface, the material must avoid the primary adhesion of living planktonic microbial cells from the surroundings. In general, this can be achieved by either repelling or killing the approaching cells. Repelling microbes have been accomplished with hydrogel coatings mostly based on PEG or similar hydrogel forming polymers, by highly negatively charged polymers or ultrahydrophobic modifications. Plasma based strategies to limit bacterial fouling of surfaces can incorporate antifouling polymer-like coatings to provide steric resistance to physical attachment of bacteria. Examples of plasma deposited polymers that have been shown to reduce short-term bacterial attachment include PEG[89] and peptide mimetic polymers.[106,107]

Kleinen et al. reported an in vivo study of the application of urological implants covered with amorphous carbon coatings optionally functionalized with O–, N– and F– containing groups.[108] This study demonstrated that differently functionalized amorphous carbon coatings are able to prevent encrustation and to reduce stent-related side effects and discomfort, whatever the plasma approach used. Apart from hydrocarbon coatings also plasma deposited organosilane[109] and chlorinated ones[110] can be effective against bacteria. Chlorinated plasma polymers with a chlorine to carbon ratio, Cl/C, higher than 1.5 can be used as an efficacious antibacterial device against *S. epidermidis* with a mechanism of action that is neither purely "leaching" nor "contact killing" but a combination of two.

Other examples of antibacterial coatings include plasma coatings containing quaternary ammonium[111,112] and pyridinium.[113] Positively charged plasma polymers based on quaternary ammonium compounds (QAC) are effective against both Gram positive and Gram negative bacteria, acting as "contact-killing coatings": extremely high electrostatic forces disrupt bacterial cell membranes by yielding removal of anionic lipids.[114,115] Plasma polymerization can difficultly achieve a QAC-like surface in one step, but, for instance, Jampala et al. have shown that an amine-rich plasma-polymer form hexamethyldisiloxane and ethylene diamine becomes quaternary ammonium rich after its immersion in hexyl bromide.[116] This solution reduced the growth of *Klebsiella pneumoniae* bacteria onto coated stainless steel substrates compared to untreated samples.

Inorganic plasma deposited coatings can be used as a valid alternative to organic ones. As an example, titanium dioxide (titania, TiO_2) is undoubtedly the most commonly employed as an intrinsically antibacterial coating thanks to its high photoactivity and stability, relatively low cost, and nontoxicity.[117,118] In this case light, oxygen and water are the only required ingredients for antimicrobial activation. Titania coatings can be either plasma sputtered from Ti or TiO_2 targets,[119] or deposited by PE-CVD of titanium containing compounds like titanium tetra-isopropoxide (TTIP, $Ti(OC_3H_7)_4$).[120,121]

Bioactive antibacterial materials

Surface coatings may also contain active components capable of killing bacteria through direct contact or via a leachable compound.[122] The concept of the polymeric spacer effect presumes that a surface grafted biocidal polymer might be capable of penetrating the bacterial cell wall of adhering bacteria. If it reaches the cytoplasmic membrane, the cell can be killed by disruption of the phospholipid bilayer. Further, surface grafted polymers are presented in such a high concentration to an adhering cell that they might even kill microbes that are not very susceptible to those polymers in solution.

As described in "Biomimetic Surfaces" section, plasma processing can be considered for surface conjugation with antibacterial compounds, following the steps depicted in Fig. 6. Antimicrobial peptides are cationic polypeptides, 12–50 aminoacids in length, produced by all organisms as a major part of their nonspecific defenses against infections. They have good activities against most bacteria and excellent activities (minimal inhibition concentration of 1–4 μg/mL) against bacteria characterized by high resistance such as multidrug resistant *Pseudomonas aeruginosa*, methicillin-resistant *Staphylococcus aureus* and *Stentrophomonas maltophilia*.[123]

Duday et al. reported the immobilization of nisin onto stainless steel via a plasma-polymerized amino-silica layer deposited via a DBD afterglow process.[124] In this case, an afterglow plasma process allowed a good compromise between stability of the plasma coating and a retention of amino-groups in sufficient density for the conjugation with nisin. Such modified materials could reduce the population of Gram positive bacteria cultivated on its surface by four orders of magnitude compared to untreated substrates. Camporeale et al. succeeded in coupling dispersin B (DspB) onto stainless steel substrates exploiting the deposition of an epoxy-rich coating using a DBD fed with glycidyl methacrylate.[125] The reduction of the adherent population of biofilm-forming *Staphylococcus epidermidis* compared to untreated substrates reached 84%.

Using similar strategies, also cationic biopolymers, such as chitosan polysaccharide, have been immobilized onto functionalized plasma-polymers; details are provided in references.[126,127]

The application of commercial antibiotics such as penicillin and vancomycin onto a plasma-functionalized surface is an obvious route towards the achievement of an antibacterial effect. However, antibiotics may not offer the convenient amine and carboxylic acid groups for the covalent attachment to plasma-modified surfaces. Hence few papers report covalent grafting of antibiotics via plasma, often via molecular spacers (polyethylene glycol in particular) to connect plasma-functionalized surfaces and antibiotics.[128–131]

Biomaterials delivering antimicrobials

Release-based coatings exert their antibacterial activity by leaching loaded antibacterial compounds over time, which allows killing of both adhered and adjacent planktonic bacteria. The release of incorporated antibacterial agents is achieved by diffusion into the aqueous medium, erosion/degradation, or hydrolysis of covalent bonds.[132] Compared with systemic methods, direct elution from the material surface offers the possibility to deliver a high antibacterial agent concentration locally, where needed, without exceeding systemic toxicity, thus minimizing the development of resistance (compare with session "Drug Delivery Systems").

Plasma processing can be used to functionalize material surface to enhance metal compounds or nano-particles (NP) adsorption from a solution/suspension, or to modify the surface of the adsorbed agent (i.e., chemical reduction, production of NPs).[133] Leys et al. described an approach consisting of a AP deposited silicone-like thin film followed by dipping-adsorption of silver nano-particles (AgNPs), and successive deposition of silicone-like barrier layer to control the Ag^+ release. Such coatings presented antimicrobial activity against *P. auruginosa*, *S. aureus*, and *C. albicans*.[134,135] A similar strategy was exploited on nylon plasma-treated with a DBD in atmospheric air.[136] An alternative consists in using hydrogel-like plasma deposited coatings to fix Ag^+ ions from a $AgNO_3$ solution.[104]

The biocide agent can be even directly deposited by a plasma process. Three main methods can be identified:

(i) PE-CVD where the precursor is a complex of the metal of interest or organo-metal compound, which exhibits a bactericidal and/or bacteriostatic activity.[137,138]

(ii) Aerosol assisted AP plasma co-deposition of an organic coating and metal nano-particles or organometal compound that can produce AgNPs (compare with section "Drug Delivery Systems").[139–141]

(iii) Simultaneous sputtering of a target coated with the antibacterial agent (i.e., Cu, Ag, TiO_2) and PE-CVD of an organic precursor.[142,143]

The latter is one of the most widely studied in this field, and, typically, leads to the formation of nanoclusters, whose size depends on the metal (oxide) content, embedded in a matrix. Silicone-like/Ag, silicone-like/Cu, diamond-like/Ag, ethylene-like/Ag parylene-like/Ag, and PEO-like/Ag nano-composite coatings have been produced with this technique.[144–146]

Nitric oxide releasing systems can be included in this kind of coatings. They exert their bactericidal activity by producing/releasing nitrogen monoxide (NO), a potent bactericidal natural molecule with pleiotropic functions, normally produced by leukocytes and implicated in innate immunity host defenses against microbial pathogens. NO can interact with superoxide, which is usually produced in tissues in conditions of oxidative stress, generating the highly cytotoxic and cytostatic peroxynitrite ($ONOO^-$).[147] Griesser et al. deposited stable plasma polymer coating, using isopentyl nitrite as precursor, which releases nitric oxide at bacteriostatic concentrations, when in contact with water, inhibiting bacterial growth without cytotoxic side effects to human mesenchymal stem/stromal cells.[148]

Micro and nano-structured materials as antibacterial surfaces

In an era of increasing antibiotic resistance, physical-derived solutions to control bacterial colonization by modification of existing implant materials presents a promising and attractive alternative to antimicrobial agents based solutions. Of particular interest is the influence that surface topography (at both nano- and micro-scale) has on bacterial viability. Two of the most widely studied physical-based strategies for antimicrobial surfaces using micro-[149] or nano-[150,151] topographies are inhibition of microbial attachment (antibiofouling) or contact-killing. A microtextured antibiofouling surface is the Sharklet micro-pattern one, inspired by shark skin.[152] When patterned onto endotracheal tubes, it was found to inhibit attachment of numerous pathogens by up to 99% compared to un-patterned controls, and also inhibited the formation of methicillin-resistant *Staphylococcus aureus* and *Pseudomonas*

aeruginosa biofilms.[153] Ivanova et al. have demonstrated that nano-features produced on cicada (Psaltoda claripennis) fly wings were extremely effective in killing Gram negative and Gram positive bacteria.[154] Following this strategy Fisher et al. proposed a microwave PE-CVD from a mixture of CH_4/H_2, followed by bias-assisted reactive ion etching.[155] These surfaces, characterized by diamond-like 3–5 μm tall nano-cones, resulted in significant *P. Aeuruginosa* killing.

An oxygen plasma treatment has been used by C. Serrano et al. on commercially available medical sutures, applied for subcutaneous implantation into 30 female, 6–8 week-old CD-1 mices.[156] Fluorescence imaging showed a significant reduction of *Escherichia coli* density at the suture but bacteria were not disrupted. In vivo tissue response of such nano-structured sutures showed that the higher aspect ratio of the nano-topography at higher etching ratios facilitates penetration into the tissue.

Plasma Processes for Tissue Engineering

The history of TE started in this scenario more than 30 years ago with the first embryonic idea to fabricate human tissues. Then, Langer and Vacanti in 1993s made it finally concrete.[32] A new discipline of the human healthcare was born involving medicine, biology, materials science, and engineering. Even far more complex as initially seemed, in the following decades it was more and more clear that Tissue Engineering was a valid alternative to the conventional organ transplantation and tissue reconstruction methods in case of tissues disease or injury.

The idea behind tissue engineering approach is in the three-step pathway sketched in Fig. 8. First, healthy cells are harvested from the patient and expanded in vitro; in a second step such cells are seeded in proper culturing conditions on 3D supports able to mimic the extracellular matrix and to allow the transport of oxygen and nutrients, as well as that of metabolites far from the construct; lastly, when cells have properly colonized the whole construct, it can be implanted into the host district or organ to give origin to newborn tissue.[31,157,158]

Today the scientific community is strongly interested in optimizing the artificial 3D structures in order to fully mimic the natural cell support in the host organisms. A wide range of both natural and synthetic materials is available, but, independently on the chosen material of natural or synthetic origin, it still needs to be processed into a suitable 3D structure, that well resembles a natural extracellular tissue environment.[32]

Nowadays the development of 3D scaffold with proper structural, chemical and surface properties is a key activity in Tissue Engineering. For a successful implantation, scaffolds must be characterized by: (i) highly porous structure with suitable interconnectivity, to guide fluids, solutes and cells transportation through the construct; (ii) mechanical properties fitting with the host tissue site; (iii) proper surface chemistry and topography, as cells interact with scaffolds primarily through its surface; (iv) degradation and resorption times matching the growth of the newborn tissue.[32,159]

As an example, Fig. 9 shows two Micro-computerized tomography (Micro-CT) images of two kind of scaffolds fabricated in Hydroxyapatite (HA) (Fig. 9A) and in poly-D,L-lactic acid (PLA) (Fig. 9C) with a focus on their open porosity (Fig. 9B and D).[160]

Plasma processing of scaffolds for TE were investigated at low pressure, but the studies revealed that a controlled modification through the whole 3D structure is not straightforward as the diffusion of the plasma active species through the scaffolds is limited by the micro-/nano-porous morphology of the polymer.[83,161–163]

In the past two decades, the research shifted toward AP plasma processes as their technology became more reliable for surface modifications, eliminate the cost of extensive vacuum equipment and are less time-consuming. Hence, many papers have been published on this topic, where the use of DBD and APPJ plasma sources is described for the chemical modification of scaffolds. Yilidrim et al. in 2008 reported the effects of O_2 fed DBD treatments on PCL supports on the cell attachment and proliferation of mouse 7F2 osteoblast cells. The hydrophobicity of the material surface was reduced and its roughness increased resulting in a

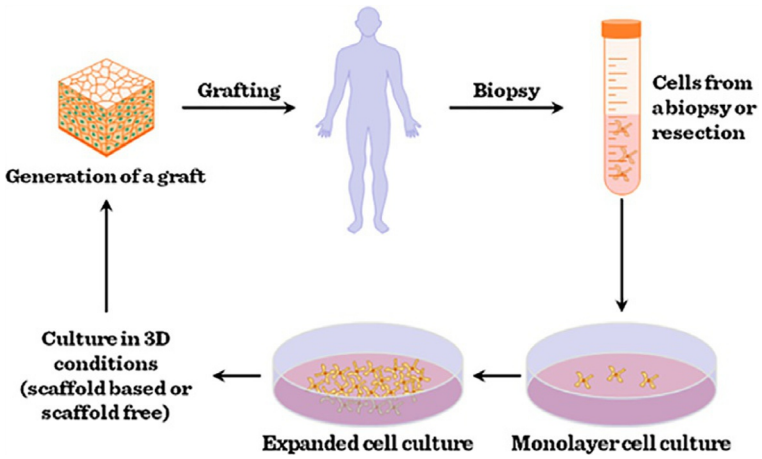

Fig. 8 Schematic representation of the tissue engineering approach. Reproduced from http://www.tankonyvtar.hu/en/tartalom/tamop425/0011_1A_3D_en_book/ch01.html.

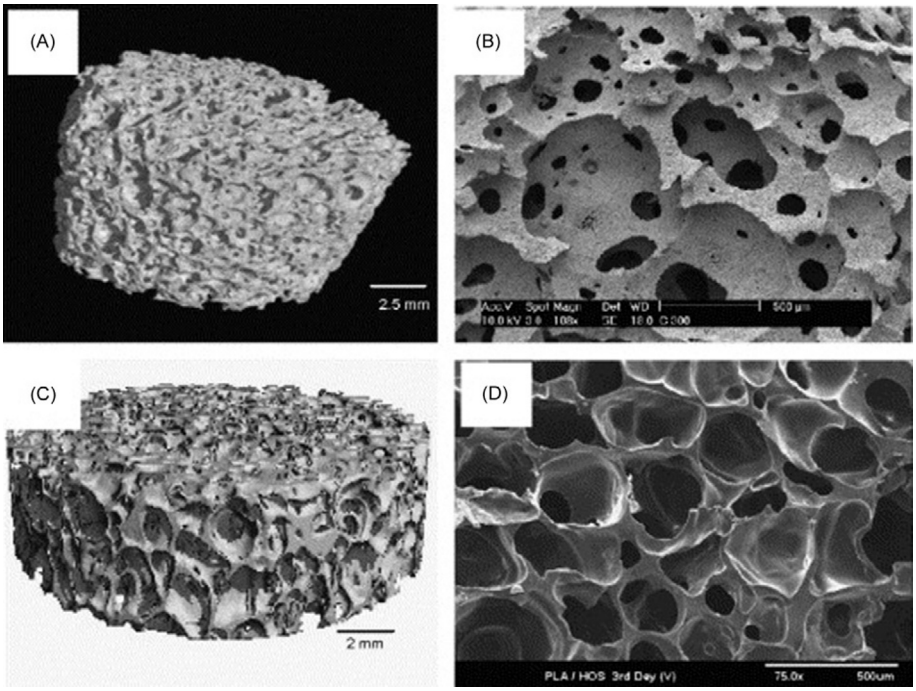

Fig. 9 Micro-CT images of a 3-D porous HA (A) and PLA scaffold (C). Corresponding SEM micrographs demonstrating a random, interconnected porous structure throughout the HA (B) and PLA scaffold (D). Reprinted from Copyright (2006), with permission from Elsevier Ltd. Silva, M. M. G. C.; Cyster, L. A.; Barry, J. J. A.; Yang, V.; Oreffo, R. O. C.; Grant, D. M.; Scotchford, C. A.; Howdle, S. M.; Shakesheff, K. M., Rose, F. R .A. J. *Biomaterials* **2006**, *27*, 5909–5917.

significant improvement of cell proliferation.[164] An interesting study on 2006 aiming at elucidating the interaction between MC3T3-E1 cells and carbondioxide (CO_2) and perfluoropropane (C_3F_8) fed APPJ modified poly-L-lactide (PLLA) surfaces has been published by Nakagawa et al.[165] The performances of PLLA samples treated with CO_2 APPJ in term of cell adhesion were enhanced with respect both untreated and C_3F_8 plasma treated samples.

In the attempt to improve the treatment of the internal surfaces of porous scaffolds, Safinia et al. described a study where air APPJ treatments of Poly(D,L-lactide)-*co*-glycolide (PLGA) copolymer scaffolds were carried out in a pumping system to force diffusion of plasma species through the scaffold. The plasma treatment enhanced the wettability at the top and at the bottom of scaffolds, as indicated by the decrease of the water contact angle. XPS demonstrated that different oxygen containing moieties were grafted at the surface of polymer substrates by APPJ.[166] More recently J.H. Lee et al. investigated air APPJ treatment of HA scaffolds for alveolar bone affected by periodontal diseases, and they were able to increase the loading of dexamethasone, an antiinflammatory drug, with the purpose of increasing osteoblast cells adhesion.[167] The same authors tested air APPJ treatments of titanium disks for enhancing the attachment of human gingival fibroblasts (HGF) and consequently reducing the osseo-integration failure of the implant. APPJ treated specimens resulted more hydrophilic due to the increase in polar groups and cell attachment resulted 20% increased.[168]

Drug Delivery Systems

Drug delivery systems allow for local administration of the substance in the place (tissue/organs) where it is really need, limiting the adverse effects of systemic approach, for which different regions of the body can interact with. An ideal drug-delivery system should possess two main characteristics: the ability to target and to control the release of the drug. Drug should target a specific site reducing side effects, this is particularly important in cancer therapy, where healthy cells should not be altered by the drug. On the other hand drugs should be released in a controlled way in terms of kinetics, to limit unwanted accumulation of the active substance, or, its limited supply.[169]

The role of plasma processing in this field can be played basically in two directions:

- deposition of a coating which acts as a partial barrier to the delivery of a drug previously casted on a surface, hence limiting the kinetic release
- direct deposition of a composite coating containing the drug.

Vasilev et al. used the first approach to sandwich levofloxacin, a common antibiotic, between two heptylamine plasma polymers layers.[170] The first coating allows for better spreading of the casted drug on the support, while the top one acted as a barrier layer

controlling the kinetic release. They tested such multilayer against *Staphylococcus aureus* and demonstrated an inhibition of the growth of bacteria and biofilm formation up to 18 h.

The same authors proposed the loading of vancomycin onto porous alumina, by immersion in a solution of the drug, and subsequent coating of the membranes with an allylamine plasma polymer. This mostly decreases the dimension of the pores at the surface and thicker coatings lead to slower release. In particular, the drug is completely released from uncoated membrane, within 45 min; depositing the plasma coating for 120 s extended the release to about 500 h.[171]

A similar approach can find application even in 3D structures like PCL scaffolds. Canal et al., optimized the plasma deposition of a PCL-*co*-PEG copolymer onto a Simvastatin (potentially osteogenic and angiogenic) loaded scaffolds. Also in this case the release of the drug was modulated, depending both on the thickness of the layer deposited, and on the morphology of the foam itself.[172]

Besides plasma deposited composite coatings delivering metal ions, described in section "Antibacterial, Antiviral and Antifungal Surfaces," recently a one-step approach has been developed, consisting in delivering a solution/suspension of the active agent in form of an aerosol into a Dielectric Barrier Discharge through an atomizer, as illustrated in Fig. 10.[13,173,174] When the feed passes through the discharge the precursor is plasma polymerized forming the matrix, and the active compound is simultaneously embedded in the growing film. Since the composition of the matrix and of the coupled active substance and their relative amount, can be tuned, depending on the specific technological requirements, this approach can be considered quite versatile. When the atomizer is supplying a biomolecule or a drug its damage is limited, because of the mild plasma conditions used and of the formation, during the aerosol generation, of a thin solvent shell all around the solute protecting it.[175]

Palumbo et al. developed this concept to prepare Lysozyme containing antibacterial composite coatings, where the precursor for the matrix was ethylene.[176] The release of the protein into water was proved and the biological activity of the embedded lysozyme as well, against *Micrococcus lysodeikticus* ATCC 4698 assessed by the agar diffusion test. Interestingly, the same authors, for the first time, evaluated possible alteration of the protein during the plasma process by means of MALDI-TOF. It was found that some limited oxidation of methionine or tryptophan residue could result, not enough to alter the activity of the embedded lysozyme.

A very similar process was considered by Da Ponte et al. to deposit PLA-like coatings containing elastin a protein typically present in connective tissues (skin, ligament, and arteries).[177] Amorosi et al. showed the possibility of designing plasma polymer films made of methacrylic acid and ethylene glycol dimethacrylate embedding acetaminophen.[178] A combination of different analytic tools indicated that the functional groups of the film are well preserved with respect to the monomer structure and that the drug is embedded in the plasma film with its structure being preserved. Furthermore, it is demonstrated that the drug can be released for up to 20 h.

Plasma Processed Micro-/Nano-Particles for Biomedical Applications

Due to their high surface area and unique physical and chemical properties, nano- and microparticles are a promising material for a variety of biomedical applications. Polymeric and inorganic particles, carbon nanotubes (CNT) and alike are widely used as drug delivery systems, biosensors, imaging devices and materials for cells growth.[179–182] Very often, before use, powders need an adequate surface treatment in order to acquire new surface properties depending on the applications, and plasma technology has proved to be a valid alternative for producing functionalized particles compared to common wet chemical methods,[183–185] However, some concerns can be encountered, when dealing with powders, because of their tendency to aggregate and of the large surface area/unit mass, leading to problems in terms of samples handling and treatment homogeneity on large scale. As a matter of fact, *ad hoc* plasma reactor geometries have been developed, both at LP and AP, requiring often an adequate stirring system, in order to expose their entire surface area to the plasma and, in turn, to achieve homogenous treatment or coating process.

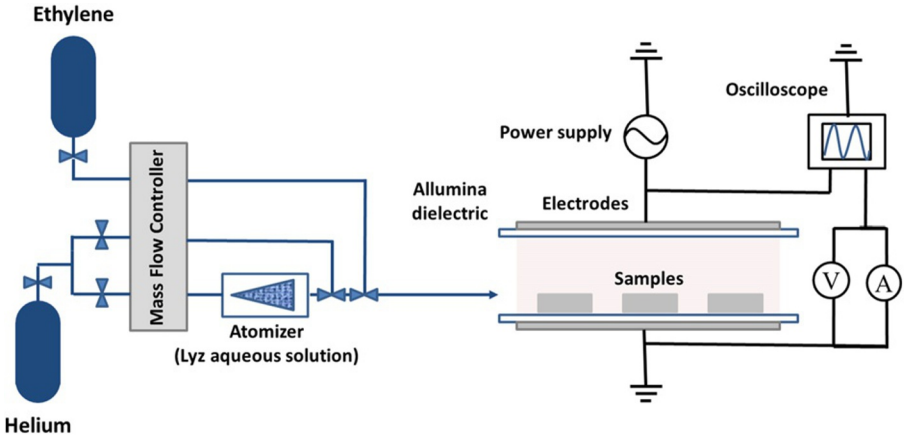

Fig. 10 Scheme of a typical equipment for plasma deposition by aerosol assisted DBD process. Reprinted from Copyright (2015), with permission from John Wiley & Sons, Inc. Palumbo, F.; Camporeale, G.; Yang, Y.-W.; Wu, J.-S.; Sardella, E.; Dilecce, G.; Calvano, C. D.; Quintieri, L.; Caputo, L.; Baruzzi, F.; Favia, P., *Plasma Process. Polym.* **2015**, *12*, 1302–1310.

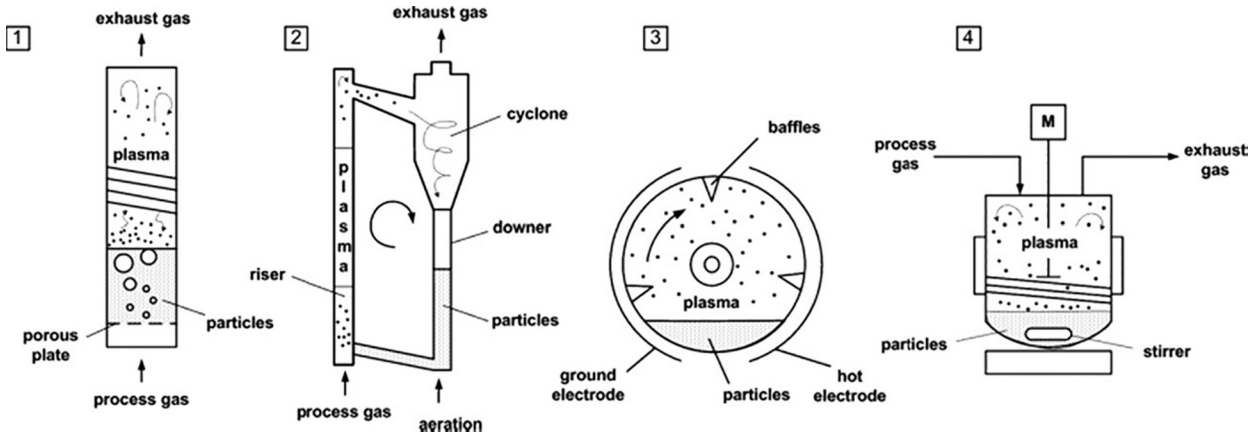

Fig. 11 Schemes of plasma rectors for powders treatment: (1) plasma fluidized bed reactor, (2) plasma circulating fluidized bed, (3) plasma rotating drum reactor, (4) plasma batch reactors. Reprinted from Copyright (2005), with permission from John Wiley & Sons, Inc. Arpagaus, C.; Sonnenfeld, A.; von Rohr, P.R., *Chem. Eng. Technol.* **2005**; 28, *1*, 87–94.

The different reactor configurations can be classified according to their plasma configuration, as reported in Fig. 11.[186] The most common plasma reactor for powders processing is the *plasma fluidized bed reactor*, where the particles are mixed in the plasma phase by the process gas flowing through.[187] The advantage of this system is the thorough mixing of particles with the plasma species. However, the amount of powders processed is limited, to attain uniformity. More uniform processes can be achieved in *plasma circulating fluidized bed reactors*, where, thanks to a cyclone unit, the powders are circulated several times in order to obtain a narrower residence time distribution and more uniform surface treatment.[188,189] Another common system is the *plasma rotating drum reactors*: particles are mixed in a rotating vessel inside which the plasma is ignited.[190,191] This set up presents the advantage of higher powders loading, and it can be used when the particles to be treated are too heavy and their fluidization in a fluidized bed reactor would be very poor. However, it can lead to heterogeneous treatments, especially for fine size particles or with a viscous behavior. Also some *plasma batch reactors* have been proposed where particles are mixed with magnetic or vibrating stirrers.[192]

Recently multiwalled CNTs have been functionalized with amine or carboxyl functional groups, exposing them respectively to He/NH_3 and humid air, by a DBD plasma, in order to generate compatible interface for enzymes immobilization.[193,194] McInnes et al. reported that biodegradable porous silicon microparticles, loaded with the anticancer drug camptothecin and then plasma coated with a hydrophobic teflon-like overlayer, could be used as drug carrier with controlled release kinetics.[195] Nonphotocatalyic anticancer effect of surface-functionalized TiO_2 nano-particles on different cell lines was examined by P. Thevenot et al. Particles were covered, using a rotating reactor, with a thin coating from plasma fed with an ethyleneglycol vinyl-ether, allyamine, and vinyl acetic acid, providing surface rich in —OR, —NH_2, and —COOH, respectively.[196] Particles can be plasma treated after depositing or growing them on a substrate as vertically-aligned CNT arrays treated with Ar plasma in order to act as a viable platform for the control and cultivation of bacterial biofilm.[197]

It should be mentioned that, in recent years, interest is growing, not only for modifying the surface of available particles, but also for developing plasma systems able to promote and control the synthesis of micro/nano-particles. Just to cite few examples, the synthesis of highly oriented silicon nanoparticles has been proposed with LP inductively coupled RF plasma,[198] atmospheric plasma of organometal compound has been investigated for the synthesis of metal NPs,[199,200] and plasma processing of liquid-phase has been developed for the synthesis of silicon and metal NPs.[201]

Sterilization and Decontamination of Materials and Devices

Since the half of the 20th century, AP plasma technology has been exploited for many environmental and industrial applications. The biomedical industry has taken particularly advantage from this technology, because of the need for the sanitation and the control of surface bacterial contamination of biomaterials.[3]

Before the advent of plasma-based techniques, the sterilization of biomedical tools was obtained either autoclaving the devices at high temperatures and high pressures or by using ionizing radiations or toxic gaseous and liquid compounds (e.g., ethylene oxide, ozone, chlorine, etc.).[202–210] Although effective, all these methods exhibit several drawbacks that made them far from being optimal approaches to sterilization, for example, the production of subproducts and residues that are toxic for humans, or they can damage the treated materials.

The first investigation on the use of plasma for bacterial inactivation dates back to the mid-1900s, when the first prototypes of ozonizers for water decontamination and destruction of environmental microorganisms were built up.[211]

The biocidal effectiveness of plasma resulted in a short while useful also for the sterilization of many biomedical devices used in the nosocomial practice, and for wound healing.[212–217]

The biocidal effect of plasma on microorganisms is basically played by UV radiations, inducing irreversible lesions on the genetic material (DNA/RNA), by chemically reactive radicals (e.g., O, OH, N), and by metastable-state atoms and molecules, with or without the assistance of ions. Chemical active species inflict structural damage affecting vital metabolic functions of the microorganisms, mostly through erosion (etching) and/or oxidation of cytoplasmic membrane.[29] The mechanism of action of plasma against bacterial cells strongly depends on the feed gas used to ignite the discharge. For instance, in humid air plasmas, antibacterial effects seem to be driven by NO and OH radicals etching the microorganisms and inducing lethal surface lesions.[218] In DBDs fed with N_2/N_2O mixtures, instead, the effect of UV radiations overwhelms the one of chemical oxidants.[219] In addition, in analogy to what happens when a pure electric field is applied to living matter, the electroporation phenomenon of the cell membranes has also been observed after plasma exposure.[220–223]

Nowadays, many certified plasma-based sterilizers are commercially available for sterilization of medical instruments. Commercialized in 1993, STERRAD® (ASP, Johnson & Johnson) was the first sterilization device successfully introduced in healthcare services and industrial sterilization. The sterilization process couples the LP, low temperature plasma technology to the bactericidal and fungicidal action of vaporized hydrogen peroxide solutions. It provides an efficient sterilization for heat- and moisture-sensitive equipment in both gastroenterology and respiratory endoscopy clinics, only producing water and oxygen as by-products.[224]

Plasma Medicine

As discussed above the advent of AP plasmas, was accompanied by its strong exploitation in biomedicine.[31] The reason of the interest is strictly related to the appropriate features of such technology. Essential is the potential of AP plasma to achieve a high gas-phase reactivity without increase of temperature, hence compatible with living tissues. Not only, AP plasmas can be very localized in the space resulting in an accurate targeting of the surface or cavity to be treated. As a consequence, it is easy to understand how fast the growth of AP plasmas has been for thermally unstable and inhomogeneous targets difficult to treat with chemicals or heat.[32]

Today plasma medicine is defined by the scientific community as a multidisciplinary field combining plasma physics, medicine and life sciences focused on the use of plasma technology in the treatment of living cells, tissues and organs, in vivo, ex vivo, and in vitro.[221] Certainly, significant progress has been reached in such field thanks to the strong efforts spent for in vitro fundamental studies.

Since the mid-1900s, DBDs first and plasma jets later on were investigated in material of safety assessment, sanitation, and bacterial control. But it is only in the early 2000s that the first investigation of plasma effects on mammalian cells have been performed. The first in vivo test of anticancer effectiveness of AP plasmas was done in 2010 by Vandamme.[225]

Thereafter, AP plasmas seemed to be effective in many different types of cancer indicating that the treatment can be uniform and not restricted to a particular tumor type. Plasma medicine has been addressed mainly to cancer therapy, wound healing and dental cure, and they will be reviewed in the following.[226,227]

Direct and *Indirect* Plasma Treatment

Strictly speaking, most of the medical applications of plasmas fit into two main categories, *direct* and *indirect* plasma treatment. There are two different approaches to define the meaning of direct/indirect.[32]

According to one approach the classification relates to the type of contact of the plasma with the target sample to be treated: (i) *direct* treatment is obtained when living tissues or organs are one of the electrodes, hence, directly participating in the active plasma discharge process. Such configuration leads to a flux of various active charged and uncharged species and UV radiation as well hitting the surface of the tissue; (ii) in contrast, in *indirect* techniques plasma and living matter are not in contact hence only uncharged atoms and molecules are delivered to the surface through the plasma.[228]

The second approach,[221] arising from more in vitro fundamentals studies, defines *direct* and *indirect* methods as follow: (i) the former consist in the exposure of the living target to the plasma source to attain a consequent therapeutic effect on it; (ii) the *indirect* mode is when a plasma-based technique is used to treat a "medium" that is then placed in contact with the living target. For example, bacteria inactivation or tumor eukaryotic cell killing can be achieved by plasma treating the physiological saline solution or culture medium and then applying such modified media to bacteria or cells, respectively, to induce a precise response.

In this sense, numerous experimental findings have revealed the crucial interaction process between the liquid and plasma. It is well documented that a key role is played by RONS and their products in both in vitro and in vivo experiments. In general, independently of the different AP plasma sources the working conditions and the different biological liquids investigated, hydrogen peroxide (H_2O_2) as well as nitrite (NO_2^-) and nitrate (NO_3^-) are detected as stable RONS. These two species are the most representative in cascade reactions where other more reactive and short-living reactive oxygen and nitrogen "precursor" species participate in, likely playing dominant roles in biological effects. Hydroxyl radicals ($^{\cdot}OH$), superoxide anion radicals ($O_2^{\cdot-}$), singlet oxygen ($^1O_2$), and nitric oxide ($^{\cdot}NO$) have been identified and partially detected in different plasma treated liquids as possible precursors. Above all, peroxynitrite ($ONOO^-$) identified by Lukes et al., seems to play a crucial role for biological effects of plasma-treated liquids.[229]

Several biological effects seems not dependent on direct plasma-cell or plasma-tissue interaction itself but more on the oxidative chemistry triggered by the plasma exposure of the liquid naturally present in in vivo targets or used for in vitro culturing.[230]

Hence, both in direct and indirect methods, plasma activation of the liquid represents the key vector of the modifications induced by the discharge. Moreover, the composition of the liquid exposed to plasma is a discriminating player in generating the chemical species responsible for the biological effects.[231]

Wound Healing

At present, a consistent part of the research is concentrated in a variety of therapeutic applications. Among these, in dermatology, especially skin disinfection/antisepsis, treatment of infectious skin diseases and wound healing are of a great interest.[232] Such important branch of plasma medicine arose from the studies onto AP plasma inactivation of microorganisms: since plasma decontamination effects on heat sensitive surfaces without damages was known, the idea of plasma processing human skin or wounds was almost consequent. In wound disinfection and healing field, a number of plasma sources has got the EC mark as medical devices during the last years and are to date commercially available. Among these, kINPen® Med (neoplas tools, Greifswald, Germany) reported in Fig. 12,[232] is featured with a helium fed plasma jet, currently successfully applied in medicine and it is considered an effective treatment method for infected, poor healing wounds, and pathogen-induced skin diseases. Bioweld1™ (IonMed, Yokneam, Israel) is ideally suited for wound and surgical incision closure and skin graft fixation.[233]

Cancer Treatments

In plasma medicine, many efforts have been dedicated at cancer care in terms of both fundamental in vitro as well as in vivo studies.[31] What is known at present is that conditions can be found that cancer cells appear more susceptible to plasma with respect to their healthy counterparts. Nowadays, the stage of clinical trials on cancer suffering patients has started.[227] Very recently, Metelmann et al. presented clinical evidence of the AP plasma in surgery treatment of advanced head neck cancer affected patients. The concept was to reduce tumor bulk by conventional surgery and then treat any remaining layers potentially composed of tumor cells by plasma-jet treatment.[234]

Recent developments of sources, where the plasma can propagate into small capillary as plasma jets, have opened up interesting perspectives to treat tumors in rectal colon, lung, and pancreas with a flexible microplasma endoscope. Since plasma therapeutic effects have been found not only at the very top surfaces of the tissue but also within the bulk of tumor mass, it has been speculated that a bystander effect could be involved in propagating the plasma induced response.[235]

Dental Treatments

Oral infections, including dental caries, periodontal and intraoral diseases, are caused by bacteria and may result in tooth destruction.[236] Teeth brushing, fluoride uptake, antibiotics, and vaccines have been used as treatment for oral disease, but with limitations.[237] Heat kills bacteria, but the application of this method to living tissues is dangerous. Sterilizing agents or antibiotics are used to treat human tissues that are infected by pathogens, but this may lead to pain and antibiotic resistance. Today, multidisciplinary joint competences (physicists, engineers, and dentist surgeons) have been involved in investigating the possible application of plasma processing in dentistry. Preliminary results indicate that this approach can be highly efficient in killing bacteria in an inexpensive manner,[238] therefore, potentially this could eliminate the problems associated with use of heat and antibiotics. Nowadays, cold AP plasmas are being exploited for treating caries, teeth whitening, malodor caring but also for treating herpetic wounds and oral cancer already mentioned in the previous paragraph.[234]

Fig. 12 The kINPen MED (neoplas tools, Greifswald, Germany). Reprinted from Copyright (2013), with permission from Elsevier Ltd. from Isbary, G.; Zimmermann, J. L.; Shimizu, T.; Li, Y. F.; Morfill, G. E.; Thomas, H. M.; Steffes, B.; Heinlin, J.; Karrer, S.; Stolz, V., *Clin. Plasma Med.* **2013**, *1*, 1, 19–23.

Plasma Agriculture

Though it cannot be included in the biomedical field, plasma agriculture is strictly related to biotechnologies, and belongs to Life Science at some extent. It is an emerging field that involves almost every step going from seed to fresh products offered to the consumers. Many studies have been brought about in the treatment of soil and water for the decontamination from organic pollutant, bacteria and fungi. Moreover, plasma treatment of water results in significant change of its properties, such as pH, oxidation-reduction potential (ORP), conductivity, and concentration of ROS and RNS, thus influencing the germination and growth of plants.[239] Direct treatment of seeds and crop is a branch of great interest: being based on nonionizing low level radiation, it could activate the vitality of seed without gene mutation increasing the crop yields, as well described in[240] for O_2 plasma treated radish sprouts seeds and it can be observed in Fig. 13. Recent research activities have demonstrated the possibility to enhance the hydrophilicity of seeds surface and thus increasing the imbibition and growth speed,[241,242] to decontaminate the surface from pesticides and fungicides or from fungi and bacteria,[243] to deposit a thin film to protect the seed[244] or to delay the germination or to reduce the water up-take if the coating is hydrophobic.[30,245] Literature presents numerous works on plasma treatment conducted on fresh fruits and vegetables[246] with the aim of decontaminate from fungi and bacteria[247] even directly in package,[248] to delay degradation and in turn increasing the shelf-life of the product,[249] to deposit an active coating on the product to adsorb molecules from the product or to release molecules to it.[250] Plasma agriculture is a promising field of application of the AP plasma technology and many research is being conducted in order to scale-up the process.

Conclusions and Perspectives

When selecting appropriate surface modification approach, there are many material- and application-based considerations that need to be addressed. From processing point of view, the choice of the appropriate method is based on its compatibility with the type of biomaterial, that is, polymeric, metallic, ceramic or composite; its stability, that is, temperature sensitivity, solubility, mechanical robustness, etc.; its physical structure, for example, porosity, and dimension, for example, bulk or thin film; to name a few. Financial cost, ease of integration, and scalability of potential modification techniques also need to be considered. It is believed that plasma processes will be more and more important for biomedical and life science oriented applications in the next future.

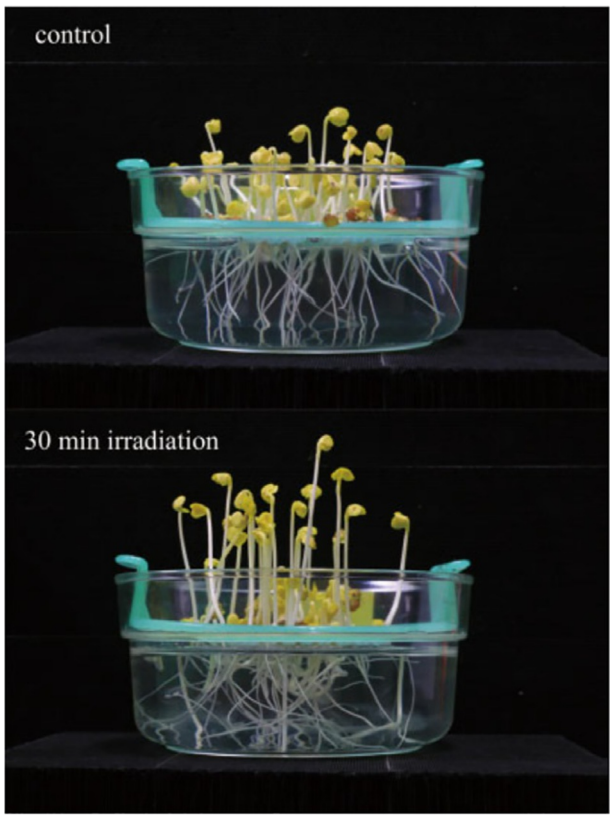

Fig. 13 Picture of radish sprouts cultivated for 7 days with and without O_2 plasma irradiation. The average length of radish sprouts was 30%–60% enhanced with plasma treatment. Reprinted from Copyright (2012), with permission of The Japan Society of Applied Physics. Kitazaki, S.; Koga, K.; Shiratani, M.; Hayashi, N., *Jpn. J. Appl. Phys.* **2012**, 51, 01AE01.

Acknowledgments

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