GAS PLASMA FOR MOLECULAR RE-ENGINEERING OF MICROFLUIDIC DEVICES

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Abstract

Technology advances in the microfluidics industry are rapidly expanding global usage of low cost Point-of-Care and companion diagnostics. Although commodity polymers meet the cost profile, they often do not meet all key performance criteria; most notably stable surface wetting of biological fluids or reagents.

Gas plasma technologies are increasingly employed to meet the demands of material selection through the molecular re-engineering of surfaces. Plasma modification using low temperature gas enables stable wetting with long shelf-life, chemical functionalization without wet chemistries, and thin film coating for promoting adhesion, barrier, or anti-fouling properties.

The work herein provides an overview of plasma surface technologies and their role in the emerging diagnostic arena.

Introduction

Theranostics (a term that combines therapeutics with diagnostics) are considered a game changing technology in the microfluidic device market. This is in part due to a shift in mindset towards patient-centric care and personalized therapy. Such devices have great potential in reducing the cost of health care while improving treatment efficacy. Past diagnostic assay used to involve specialized methods requiring expensive lab-scale equipment and/or highly trained operators.[1] Examples of products considered part of the microfluidic market are illustrated in Table 1.

The estimated market value of Point-of-Care varies however Yole[2] has projected $22.6 billion by 2016 (includes diabetes self-testing tools).

Within Point-of-Care (POC) devices there is special focus on low cost detection of disease and viruses. Many companies are competing to introduce their platforms into the exploding market.

Table 1 Examples of Microfluidic Devices

Micro Electro Mechanical Systems (MEMS)	Lab on a chip (LOAC)	Assay Tools for genomics applications
Pressure sensors	Gas chromatography devices	DNA Assays
Fluid Handling (Fluidic MEMS)	Point of Care (POC) testing	Microarrays (DNA Chips)
Fluid Storage	Diagnostic Devices	Protein Arrays
Micro-Total-Analysis-Systems (microTAS)		

The materials and processes used to manufacture detection tools demand low cost.

> "At present, monitoring the virus in patients presents a logistical challenge, even in wealthy areas. In such places, an HIV-positive patient's blood may be drawn every six months by a trained nurse and stored in $40 worth of reagents, refrigerated and sent to a laboratory where a skilled technician uses a laser-equipped machine costing around $100,000 to count the CD4 immune cells in the patient's blood. The turnaround of results could shorten to hours, rather than weeks, thanks to recent arrivals on the point-of-care market…"[3]

Companies are incorporating low cost commodity plastics into their platforms in order to replace more costly glass or silicon substrates. The latter usually require expensive and inflexible processing tools. Polymers are "becoming the reference substrate for Point-of-Care applications."[2] However most low cost polymers require performance enhancing surface modification in order to meet their service demands.

How a POC Device Works

> "Microfluidic devices are a lot like computer chips with plumbing" [4]

The basic process flow of a POC device is:

- Collect specimen - direct or stored
- Input cartridge (with special reagent)
- Read cartridge with instrument
- Output result

POC devices offer the market cost effective means of precision automation for the manipulation of small fluidic volumes.[5] Use of plastics enables lower cost and offers greater opportunities for high throughput at lower time to development.

Examples of specific POC devices include:

- Blood glucose monitoring
- Blood gas and electrolytes
- Rapid coagulation
- Rapid cardiac markers
- Substance abuse
- Infectious Diseases
- Urine Strip
- Pregnancy
- Fecal
- Cholesterol screening
- Food pathogens detection
- Hemoglobin diagnostics

Common Materials

Polymers used in the manufacturing of POC devices include:

- Cyclo-olefin polymer (COP) or copolymer (COC)
- Poly(methyl methacrylate) (PMMA)
- Polydimethylsiloxane (PDMS)
- Thermoplastic Polyolefins (PE, PP)
- Polyesters (PET)
- Polystyrene (PS)

Industry Requirements

- Low operation cost
- Optical transmission
- No spalling/coating lift
- Long shelf life
- Non-biofouling
- Inline processing
- Consistency of surface

METHODS

Gas plasma modifications are achieved atmospherically and as vacuum-based processes. The focus of this paper is on low pressure plasma applications.

The components to be treated are placed in a reactor chamber under low pressure. A vacuum pump removes air from the chamber to an average base pressure of 100 to 25 mTorr. Process gas(es) and/or liquid vapor are introduced into the chamber increasing the pressure to an equilibrium value of 500 to 100 mTorr.

Electrodes within the chamber excite the gas or vapor species using radio-frequency energy. The species are energized into a plasma (4^{th} state of matter) comprised of modest concentrations of electrons, ions, and other meta-stables. These excited species have sufficient energy to rupture the chemical bonds at the surfaces of the matter it contacts. The ruptured bonds are thermodynamically unstable and return to equilibrium through recombination with the gas fragments available in the plasma. Rapid molecular re-engineering of the surface takes place in the plasma reactor within seconds or minutes.

Cold gas plasma processes are efficient low energy processes. Bulk properties remain unchanged because the energized species have little penetrating energy, thus the modification is confined to the topmost molecular layers. The low heat generation enables surface treatment of ultra-thin and thermally sensitive materials. As practiced in non-semiconductor applications, cold gas plasma is recognized as both a worker and a workplace safe clean air technology.[6]

Plasma Reaction Chamber Configurations

The product's form factor dictates the configuration of the plasma reactor chamber and electrode. Plasma equipment designs are available for the treatment of powders, fiber, large structures, complex geometries, and roll-to-roll goods.

Examples of the primary plasma systems in-use for various applications include:

Figure 1 exhibits the Plasma Technology Systems Aurora 350 Small batch reactor chamber with side wall electrodes and changeable shelf arrangement. (3 cubic foot aluminum chamber)

Figure 1. Porous polyethylene caps loaded on trays for chemistry discovery trials.

Figure 2 exhibits the Plasma Science 0500 Tumbler reactor chamber with center pin electrode and rotating basket. (5 cubic foot aluminum chamber; approximately 1 cubic foot load capacity)

Figure 2. PS0500 Tumbler System loaded with polyethylene filters

Figure 3 exhibits the Plasma Science PS0500 system with fixed shelf electrode. (5 cubic foot aluminum chamber)

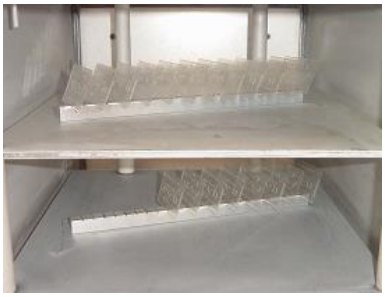


Figure 3. PS0500 system loaded with microfluidic cartridges

Figures 4 and 5 exhibit the Plasma Science large roll-to-roll system for treatment of film or membrane up to 60 inches wide. Material passes free span over multiple sets of electrode plates providing 20 feet of primary plasma zone. Line speed is determined by process conditions and substrate material. Speeds up to 100 feet per minute are attainable.

Figure 4. 60 inch roll-to-roll treatment system (show in open position for loading)

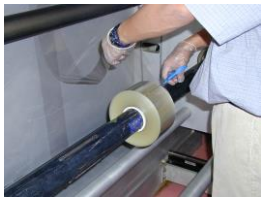


Figure 5. Operator shown removing treated film from roll-to-roll system

Example Plasma Surface Modification Applications for Microfluidic Devices

Stable Wetting

Many low cost plastics typically lack the surface polarity which makes it wettable to aqueous solution or biological reagents. Untreated polyethylene, for example, is hydrophobic consisting of only carbon and hydrogen atoms and lacking functional oxygen.

Microfluidic and diagnostic devices often make use of sintered polyethylene materials as membranes, wicks or separation filters [7]. Porous materials may also be used for surface reactions and for the capture of biomolecules. Surface modification is employed for providing both surface wettability and surface functionalization. Rapid and uniform wetting is a key characteristic for many microfluidics. Rapid absorption helps to ensure that fluid is “drawn away from the surface” to minimize contact or transfer. [8]

The hydrophobic behavior of polyethylene impacts the wicking or wetting. Wettable coatings are available through chemistry however plasma provides advantages in control, speed, and it is a single step operation. Furthermore the plasma process contains no chemical waste, rinse, or curing time.

Table 2 illustrates the elemental compositions of three polyethylene surfaces using XPS following modification with different plasma chemistries. All plasma modified surfaces now contain significant levels of polar oxygen species as compared to a theoretical 0% atomic oxygen for unmodified polyethylene. Values noted with '*' contain trace level or signal error. All elemental fractions denoted '-' had no signal detected. [9]

Table 2. XPS of plasma treated polyethylene [9]

Plasma chemistry	**C**	**N**	**O**	**F**	**Na**	**P**	**Cl**
CO_2 / CH_3OH	85	--	15	--	--	--	--
O_2 / CH_3COCH_3	80	0.4*	20	--	--	0.2*	--
CO_2 / N_2O	80	1.4	16	2.7	0.4	--	0.1*

High resolution XPS is employed for the identification of oxygen compounds. XPS data was corrected for binding energy of the $-(CH2)_n-$ signal at 284.6 eV. Atom percentages were tabulated and peak assignments were made based on the binding energies of reference oxygen compounds C1 thru C4. Table 3 summarizes the results and their respective oxygen compounds.

Table 3 High resolution XPS data

Peak Assignments:
C1 = C-R (R=C, H)
C2 = C-OR (hydroxyl)
C3 = O=C-R (carbonyl)
C4 = O=C-OR, O-CO-O, C_F (?) (carboxylic acid)

Sample Description	**C1**	**C2**	**C3**	**C4**
CO2 / CH3OH				
Binding energy (eV)	284.6	286.0	287.5	288.9
Atom Percent	69.0	8.9	3.4	3.1
O2/ CH3COCH3				
Binding energy (eV)	284.6	286.0	287.3	289.1
Atom Percent	66.0	4.3	3.7	6.2
CO2 / N2O				
Binding energy (eV)	284.6	286.1	287.1	288.6
Atom Percent	69.0	3.6	2.8	4.5

The three plasmas examined in Tables 2 and 3 readily show oxidation on the polyethylene surfaces following plasma treatment. High resolution XPS adds further insight into the precise oxygen compound that are incorporated to the surface. Fine tuning of the relative species density and loading is possible. For example hydroxyl content on polyethylene may be varied from 3.6 to 8.9 percent through judicious selection of the plasma co-reactant gas. [9]

The surface energy of the polyethylene from all of these three treatments is greater than 70 dyne. Each is readily wetted with distilled deionized water and the droplet is self-spreading. [9]

An example of the stable hydrophilization of a polyethylene membrane using plasma treatment is shown in Figure 6.

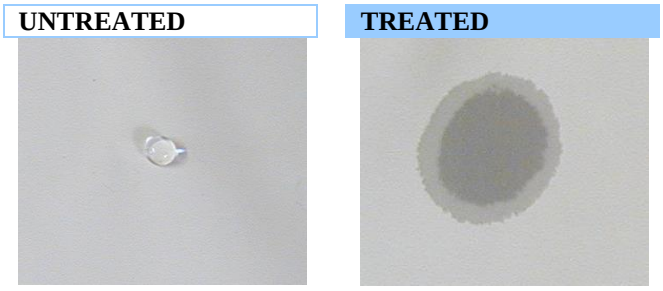


Figure 6. Demonstrating activation of polyethylene membrane.

In low pressure primary plasma the energized species are able to modify the interstices of porous media. Penetration into media is enabled by gas diffusion and the extended mean free path of a gas under vacuum pressure. Figure 7 illustrates wicking of a porous frit in a time resolved image series using 70 dyne test ink purchased from Accudyne ASTM Standard D2578.

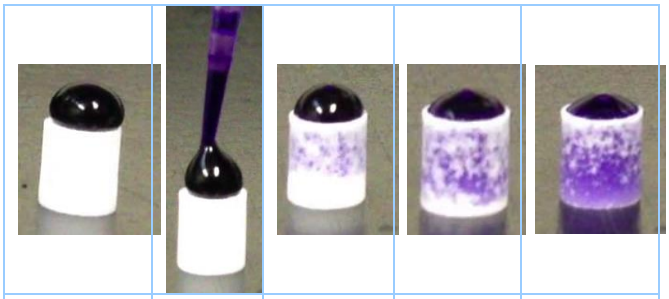


Figure 7. Wicking behavior of plasma treated polyethylene filters.

Similar processes are used for modification of COP, COC and polystyrene substrates. The utility of plasma is universal across all polymers, albeit the optimum selection of gas or process parameters may differ from one polymer system to another. With optimization of the plasma conditions these processes do provide stable wetting on most polymers. A COP film following plasma treatment exhibits a 20 percent increase in oxygen species on the surface using XPS. Stability of the oxygen incorporation is shown in Figure 8. Wetting

behavior of COC (no aging) and COP following 6 month storage is shown in Figures 9 and 10.

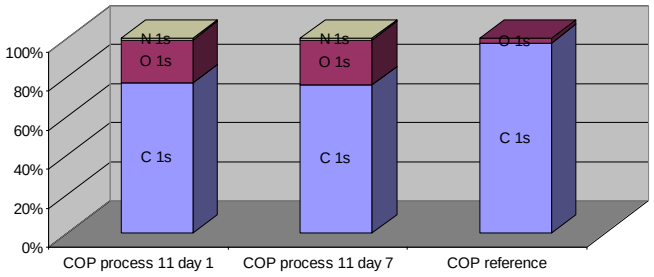


Figure 8 Plasma modified COP film after 7 days

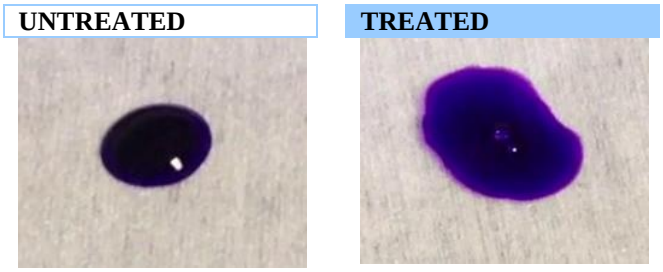


Figure 9. Plasma treated COC, no aging, tested with 70 dyne-cm fluid.

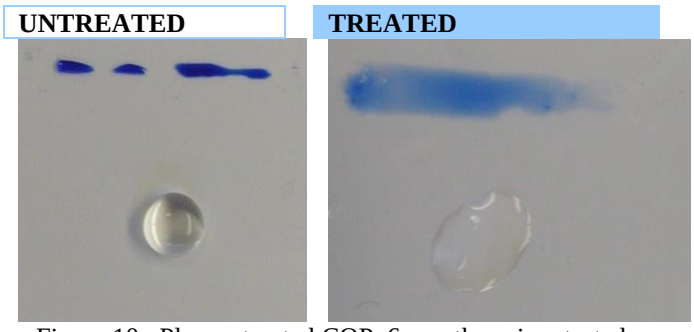


Figure 10. Plasma treated COP, 6 months aging, tested with 56 dyne-cm fluid.

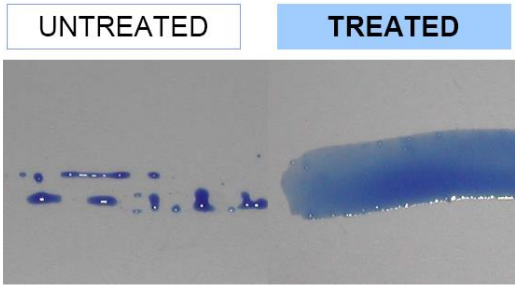


Figure 11. Plasma treated polystyrene, 18 month aging, tested with 56 dyne-cm fluid.

A similar increase in hydrophilicity is observed on polystyrene surfaces. Shelf life testing (real time aging) demonstrates the stability of the surface to wet after 1.5 years following plasma surface modification. Shown in Figure 11 is the surface before plasma and following 18 month storage. The treated surface wetted at 56 dyne-cm.

Chemical Functionality

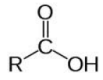
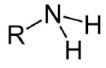
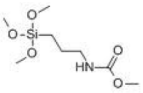
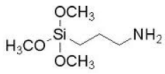
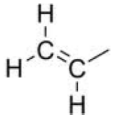
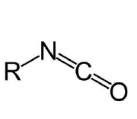
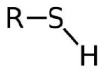
Functionalization is also known as activation. Surfaces may be hydrophilic as shown in the previous section. Through process selection a functional surfaces may be tailored with properties that may be non-polar, polar, neutral, charged, and hydrophobic.

A functionalized surface enables covalently bound:

- Polyethylene Glycol (PEG)
- Hyaluronic acid
- Polylactic acid or polylactide (PLA)
- Surfactant coatings
- Hydrogels
- Amino acids, peptide attachment
- Coatings to resist biofilm attachment
- Antimicrobials
- Biomolecular immobilizations

Common surface functionalities introduced via plasma include:

Table 4. Surface Functional Groups

		
Hydroxyl	Carboxyl	Amine
		
Carbonyl	Glycidyl	Thiol
		
Vinyl	Isocyanate	Chloride

As shown in Table 4, oxygen and oxygen containing compounds are not the only attainable products of gas plasma treatment. For example, ammonia is routinely employed to provide amine or amino functionality to a variety of plastics.

> "Plasma produced reactive surfaces with amine, carboxy, hydroxy, and aldehyde groups have been used by many scientists because of their compatibility with well-established chemical reactions for grafting of bioactive moieties such as enzymes, antibodies, proteins, and glycosaminoglycans." [10]

Griesser continues that amination is a successful method to enable covalent reactions for cellular colonization, yet functional groups may degrade over time requiring a short time between plasma and subsequent reaction. [10]

Carboxyl groups provide a more stable surface, assumed "probably because carboxylate groups are a product of post-plasma oxidative reactions anyway." [10]

A study was commenced where a variety of chemistries focusing on amine and carboxyl moieties were used to functionalize a nylon woven (25 um mesh opening) which is structure used for cellular assays. The photographs in Figure 12 display untreated and treated samples following a carboxyl functionalization.

The images demonstrate binding capability following two months of aging using a fluorescing probe in combination with a novel wash protocol. The result is preferential cellular binding with little to no non-specific interaction; no cells or debris present.

As compared to the carboxyl oriented plasma, an amination attempt to the surface provided less desirable results in this scenario. The author concluded that the amination was not stable following Griesser's hypothesis for degradation of reactivity in amines.

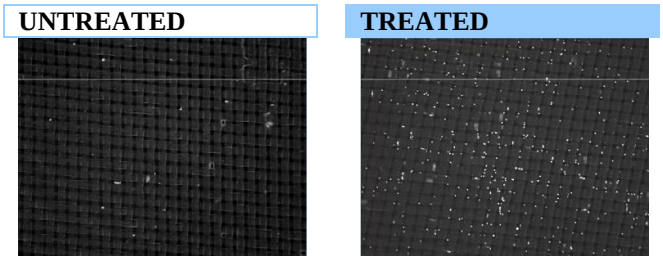


Figure 12. Biotinylated fluorescing probes bound to mesh following plasma functionalization.

Plasma Enhanced Chemical Vapor Deposition

A more complex vapor or gases such as hexamethyldisiloxane may be employed for plasma deposition onto surfaces. This process is called Plasma Enhanced Chemical Vapor Deposition (PECVD). Depending on the process parameters many compounds will deposit a coating that may resemble products obtained from conventional polymerization. Because the reaction is carried out in a closed chamber and the process parameters are completely controlled, the process is inherently reproducible and reliable. Such PECVD coatings are routinely employed to create gas and chemical barrier coatings on conventional polymers. [9]

Other example PECVD coatings include:

- Polystyrene, Polyethylene
- Fluoropolymer, fluoroacrylates
- PEGylated (Tetraglyme)
- Aminated
- Polyacrylate
- Hydroxyethyl methacrylate (HEMA)
- Ethylene Oxide

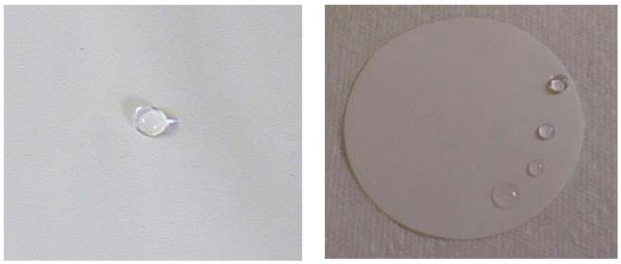
Non-Fouling Surfaces

When an inert or chemical resistant (non-fouling) surface is required, PECVD enables the deposition of nano-thin cladding that do not impact bulk nor optical properties. Plasma deposited coatings enable conformal coverage of complex geometries and they improve adhesion in challenging experiences.

A fluoroacrylate coating was deposited on a BD-Falcon PS petri dish with two process variations comparing the anti-fouling characteristics of the resulting coating to the plasma power density. To test cell adhesion the petri dishes were then plating with ckit selected human amniotic fluid stem cells. In the case of Process 1 some cells were able to adhere to the plasma coated petri dish however the cells did not spread out on the petri dish or differentiate. The cells normally flatten and begin proliferation. They were however able to divide; albeit at a relatively slower rate compared to petri dish uncoated. So the result of Process 1 is that some cells could adhere and divide but at rates much slower than a control surface. In the case of Process 2, however, cells did not adhere to the petri dish surface. As a result most or all cells eventually died. [11]

Fluoropolymer Surfaces to Control Fluid Transport

Plasma deposited coatings may be applied to a variety of substrates in a controlled method. Figure 13 demonstrates two different hydrophilic materials (a polyester and paper) that have received an ultra-thin fluoropolymer hydrophobic and omniphobic (coating via plasma deposition. Omniphobic surfaces are designed for non-specific –phobicity. The left image in Figure 13 demonstrates water, oil and alcohol –phobicity on a paper filter. The right image is a woven polyester filter. The coating enables controlled -phobicity to water, allowing other fluids to pass through. In addition to wicking of fluid, controlled fluid transport is desirable for a variety of filtration applications.



Hydrophobic Polyester Filter | Omniphobic Paper Filter

Figure 13 plasma deposited fluoropolymer coating on porous media

Bonding of Pressure Sensitive Adhesives

Plasma surface modification is also used to create reactive functionality on a surface which can be then covalently bonded to a chosen Pressure Sensitive Adhesive. Plasma provides the select chemical functionality (Table 2) that is reactive with the adhesive system. Covalent chemical bonding provides an environmentally stable and permanent bond between the adhesive film and plasma treated substrate. [12]

Conclusion

Use of low pressure RF plasma enables the researcher and engineers a tool box for providing precise surface chemistry with greater independence from a bulk material's constraints. This concerns microfluidics for achieving wetting, adhesion, bonding and surface reaction or immobilization. Myriad chemistry combinations may be introduced in a controlled manner.

The utility of plasma is universal across all polymers, albeit the optimum selection of gas or process parameters may differ from one polymer to another.

The advantage of plasma surface modification is that it creates high performance surfaces onto low cost consumable grade materials, benefitting the rapidly expanding global microfluidic markets.

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