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Influence of Oxygen Plasma Modification on Surface Free Energy of PMMA Films and Cell Attachment

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Summary: For any biomaterial placed into a biological medium, the surface properties of the material, such as porosity, crystallinity, presence and distribution of electrical charge and functional groups are very critical parameters that determine the acceptance or rejection of the material. Applications, especially tissue engineering require some surface modifications at the molecular level without disturbing the bulk properties of the implants in order to enhance the cell attachment on the material. An appropriate technique is the application of glow discharge plasma which employs no solvents, takes place at ambient temperatures, and alterations take place only at the surface by changing the surface chemistry along with surface free energy (SFE) and efficiency for cell-material interaction. In this study, poly(methyl methacrylate) (PMMA) film surfaces were modified with oxygen plasma. SFE and its dispersive and polar (acidic-basic) components of the modified surfaces were calculated by means of several theoretical approaches including geometric mean, harmonic mean and acid-base equations. The relation between SFE and its dispersive and polar components and cell attachment on surfaces were studied. The highest 3T3 cell attachment was obtained for the surface with the total SFE of 61.77 mJ/m^2 and polar component of 50.91 mJ/m^2 according to Geometric mean. The total SFE of this surface was calculated to be 61.06 mJ/m^2 and the polar component as 40.96 mJ/m^2 using the Harmonic mean method.

Keywords: contact angle; modification; PMMA; surface free energy; surfaces

Introduction

When an implant is introduced the human body, the first reactions of the implant surface determines whether it would be accepted. The first interaction of the material with its environment involves the few nanometers of the surface.[1] Attachment of cells on the material is desired in applications like dental or bone implants, but no interaction is preferred in applications like stents. Since there are many factors, such as chemistry, surface topography and roughness, presence and distribution of certain groups, surface charge and polarity, surface free energy, etc, affecting cell-material interactions; the cell adhesion scenario is quite complicated. In the absence of specific binding groups, van der Waals forces and plurivalent cation bridging drive the cells to attach to the surfaces. In the presence of an extracellular matrix the cell-material interaction is an ionic one. In their absence this interaction should lead to an equilibrium spreading (a certain contact area) which is apparent in the cell morphology. Recent studies in tissue engineering increased the importance of the optimum surface properties for cell attachment, spreading, proliferation and even differentiation. In such a study the surface of the biomaterial

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needs to be modified without altering the other properties.

Although there are various chemical or physical methods to modify the surfaces, RF-plasma treatment is a convenient one since it alters only the few nanometers of the surface without affecting the bulk properties. Plasma can be created under vacuum at ambient temperatures. During this process, the materials are placed in the reaction chamber, the system is evacuated and then the required gas is allowed in the reaction chamber and electrical discharge is created with application of certain RF-frequency. Depending on the parameters such as type of the gas, operating pressure, applied power and discharge duration; various reactions such as etching, degradation, crosslinking or coating take place.[2–5] If just oxygen gas is used, hydroxy, carboxy or peroxy functionalities are created and in general wettability of the surface increases.

The aim of this study was to modify the surface of solvent cast PMMA films by oxygen plasma, examine the changes in surface chemistry, surface free energy and its components by using different theoretical approaches, and study the effect of surface modification on cell attachment.

Materials and Methods

Materials

Poly(methyl methacrylate) (PMMA, MW0 120000 Da), diodomethane, glycerol, ethyleneglycol and dimethylsulfoxide were products of Acros (USA) and diethylene glycol was product of Fisher (USA). Tricresylphosphate and bromonaphtalene were obtained from Aldrich (Germany). Aniline and formamide were products of Merck (Germany) and chloroform was from Lab-Scan (Ireland). In all the experiments deionized triple distilled water was used. For the liquids, surface tension, dispersive and polar component as well as acidic and basic subcomponent values were obtained from literature[6–7] and are given in Table 1. 3T3 cell line was purchased from Foot-and-Mouth Disease Institute of Ministry of Agriculture & Rural Affairs (Turkey)

Preparation and Modification of PMMA Films

PMMA solutions (20% w/w in chloroform) were placed on microscope slides, dried at room temperature for 5 to 7 days and then placed in a vacuum oven for few hours at room temperature for complete removal of chloroform. These samples were placed in the plasma reaction chamber (Advanced Plasma Systems Inc., USA), and oxygen gas was introduced at a controlled rate to maintain the plasma pressure at 20 mTorr. Discharge was created using an RF generator (Seren R300 13.56 MHz, Turkey) while the impedance of the RF source eliminated the reflected power with a matching network. Different powers (5, 20, 100 or 300 Watts) were applied for either 1 min or 15 min. After the treatment,

Table 1.
Surface tensions and their components for the test liquids.

LIQUID	Symbol	Total SFE	Dispersive (γ^d)	Polar (γ_L^p)	Basic	Acidic
		γ_L (mJ/m^2)	γ^{LW} (mJ/m^2)	γ_L^{AB} (mJ/m^2)	γ_L^{--} (mJ/m^2)	γ_L^{+} (mJ/m^2)
Water[a)]	W	72.8	26.25	46.55	11.16	48.5
Water	W	72.8	21.8	51	–	–
Glycerol[a)]	G	64	35.05	28.55	7.33	27.8
Glycerol	G	64	34	30	–	–
Formamide[a)]	F	58	35.5	22.5	11.3	11.3
Formamide	F	58.2	39.5	18.7	–	–
Ethylene Glycol[a)]	E	48	33.9	14.1	51.6	0.97
Ethylene Glycol	E	48	29	19	–	–
Diethylene Glycol	De	44.4	31.7	12.7	–	–
Diiodomethane[a)]	Dm	50.8	50.8	0	0	0

[a)] *Acidic and basic components from the studies of Della Volpe and Siboni.[8,9]*

oxygen gas flow was continued for 30 min. more for the termination of the created radicals. The samples were immediately used in contact angle measurements.

Contact Angle Measurements

Test liquids (10 μL) were placed on PMMA surfaces by a micro syringe and the images of the droplets were obtained by a digital camera (Fujifilm F FX-6900 Zoom-E, Japan) in a constant temperature environment at 20 °C. Contact angles (θ) were calculated from the images by using Windows Excel and Paint programs. At least five (mostly 8) measurements were made for each sample.

Surface Free Energy Calculation

Surface free energy, and its dispersive and polar components, and acidic and basic subcomponents were calculated by using (i) geometric mean (equation 1), (ii) harmonic mean (equation 2) and (iii) acid-base (equation 3) equations. In the calculations Windows Excel and Mathpad (Mark Widholm) programs were used in the MAC OS environment.

$$\gamma_{sl} = \gamma_{sv} + \gamma_{lv} - 2\left(\left(\gamma_{lv}^{d}\gamma_{sv}^{d}\right)^{1/2} + \left(\gamma_{lv}^{p}\gamma_{sv}^{p}\right)^{1/2}\right) \quad (1)$$

$$\gamma_{sl} = \gamma_{sv} + \gamma_{lv} - 4\left(\frac{\gamma_{lv}^{d}\gamma_{sv}^{d}}{\gamma_{lv}^{d} + \gamma_{sv}^{d}} + \frac{\gamma_{lv}^{p}\gamma_{sv}^{p}}{\gamma_{lv}^{p} + \gamma_{sv}^{p}}\right) \quad (2)$$

$$(Cos\theta + 1)\gamma_{l} = 2\left(\sqrt{\gamma_{l}^{LW}\gamma_{s}^{LW}} + \sqrt{\gamma_{l}^{+}\gamma_{s}^{-}} + \sqrt{\gamma_{s}^{+}\gamma_{l}^{-}}\right) \quad (3)$$

In the equations, γ_{sl}, γ_{sv} and γ_{lv} define the interaction vectors between solid-liquid, solid-vapor and liquid-vapor, respectively. γ^{d} and γ^{p} are the dispersive and polar components. γ^{LW}, γ^{+} and γ^{-} are the Lifshitz-van der Waals, acidic, and basic components of SFE, respectively.

Cell Culture Studies

3T3 cells were cultivated in high glucose DMEM supplemented with 5% fetal bovine serum (FBS), 100 units/mL penicillin and 100 units/mL streptomycin at 37 °C in a carbon dioxide incubator (5%CO_2, MCO-17AIC, Sanyo Electric Co. Ltd., Japan). PMMA films (sterilized by UV exposure for 30 min at room temperature) were placed into 24-well plates and cells (50000 cells/20 μL) were seeded on each film. After 30 min, 500 μL of high glucose DMEM supplemented with 5%FBS, 100 units/mL penicillin and 100 units/mL streptomycin were added and incubated in a CO_2 incubator (5%CO_2, 37 °C) for 24 h. Cell numbers were quantified by MTS assay using a calibration curve. For this purpose, the samples were transferred into a different 24-well plate, gently washed with PBS, MTS solution (500 μL) was added and incubated for 2 h in CO_2 incubator. Aliquots taken from the wells were used in the determination of 3T3 cell numbers with a kinetic microplate reader (Maxline Vmax, Molecular Devices, USA) at OD 490 nm. Tissue culture polystyrene (TCPS) was used as the positive control for cell adhesion.

Imaging of the Cells

Images of the cells were obtained by using fluorescence microscope (IX 70, Olympus, Japan). For this purpose, 3T3 cells were fixed by glutaraldehyde (2.5%) for 2 h and then washed twice with cacodylate buffer (0.1 M, pH 7.4). For staining with Acridine orange, samples were washed with HCl (0.1 M) for 1 min, Acridine orange was added and after 15 min the samples were washed with distilled water. The cells were examined with a fluorescence microscope at the excitation wavelength of 480 nm.

Results and Discussions

Contact Angles of PMMA Surfaces

Contact angles of the prepared PMMA samples with various liquids are given in Table 2. Water contact angles decreased from 63.5 to 26.7 degrees as the plasma power and duration were increased from 5 to 300 Watts and from 1 to 15 min, respectively. The decrease in water contact

Table 2.
Contact angle values of different liquids on PMMA surfaces.

Sample	Contact Angle (°)					
	Water (W)	Glycerol (G)	Formamide (F)	Ethylene Glycol (E)	Diethylene Glycol (De)	Diiodomethane (Dm)
CONTROL	63.5	53.0	48.8	40.4	33.3	28.8
20 W 1 min	43.0	34.2	20.2	19.3	21.7	26.1
100 W 1 min	38.3	35.6	23.6	24.4	14.0	28.8
300 W 1 min	39.5	35.1	15.6	CW	17.7	29.2
5W 15 min	57.8	49.3	45.1	42.7	38.0	34.3
20 W 15 min	37.1	35.0	12.1	24.6	17.9	26.1
100 W 15 min	34.6	34.7	CW	9.5	11.0	22.3
300 W 15 min	26.7	44.8	CW	CW	9.7	12.0

CW means complete wetting.

angles indicates an increase in hydrophilic property due to the formation of oxygen containing groups on the surface. These groups contribute to the polar component of SFE. For all samples the same trend of decreasing contact angles were observed except for the samples treated with 5 Watts; most probably because of nonhomogeneous plasma formation in the reactor at this very low plasma conditions. Therefore these results were not considered in the calculations. For the samples treated with 300 Watt, some liquids like formamide and ethylene glycol spread on the surface resulting complete wetting.

Application of Geometric Mean and Harmonic Mean Equations

Dispersive and polar components of SFE, obtained from Geometric Mean and Harmonic Mean Equations for different liquid pairs and for 1 and 15 min treated samples are given in Table 3 and 4, respectively. These results demonstrated very wide range of values. However, the general trend shows an increase in total SFE with the applied power and also with the duration of plasma. For untreated control group, the dispersive and polar components were found to be similar and about 20 mJ/m^2 resulting total SFE about 40 mJ/m^2 for both approaches. Application of plasma caused an increase in both dispersive and polar components resulting in an increase in the total SFE to about 55–59 mJ/m^2 for 1 min, and about 56–61 mJ/m^2 for 15 min plasma applications as the power increased up to 300 watts.

The common liquid pairs, which lead results in calculations, were water-glycerol, glycerol-diethylene glycol and water-diethylene glycol. Shimuzu and Demarquette[10] previously suggested that, in Geometric or Harmonic mean methods, the most trustable results could be obtained if the pair consisted of one polar and one non-polar liquid. In the present study, water-diethylene glycol pair is the one that has the largest polarity difference among all liquids, and the results of this pair appear to follow the trend of the power used in plasma treatment. Water-diethylene glycol (W-De) pair gave acceptable results for all the samples and the change in SFE, and polar and dispersive components of SFE were calculated based on this pair, by Geometric and Harmonic mean approaches (Figure 1).

The value of dispersive component of SFE in the control group was found to be 19.27 mJ/m^2 and 19.70 mJ/m^2 using the Geometric mean, and Harmonic mean approaches, respectively. When the dispersive component was examined, it was observed that, with the Geometric Mean approach a decrease from 19.27 mJ/m^2 to 8.62 mJ/m^2 was obtained for plasma applications up to 300 W, while Harmonic mean approach did not show a significant variation; stayed almost constant at about 20 mJ/m^2.

Table 3.
Dispersive and polar components of SFE for the samples treated for 1 min.

Geometric Mean				Harmonic Mean			
Liquid Pair	Dispersive Component *(mJ/m^2)*	Polar Component *(mJ/m^2)*	Total *(mJ/m^2)*	Liquid Pair	Dispersive Component *(mJ/m^2)*	Polar Component *(mJ/m^2)*	Total *(mJ/m^2)*
CONTROL				**CONTROL**			
W-G	23,37	17,73	41,10	W-G	20,00	23,07	43,07
W-F	21,83	18,61	40,44	W-F	21,60	22,60	44,20
W-De	19,27	20,25	39,52	W-De	19,70	23,40	43,10
G-De	16,28	25,61	41,89	G-De	19,70	23,41	43,11
Average	*20,19*	*20,55*	*40,74*	*Average*	*20,25*	*23,12*	*43,37*
20 W 1 MINUTE PLASMA				**20 W 1 MINUTE PLASMA**			
W-G	20,30	34,53	54,83	W-G	21,30	34,93	56,23
W-F	27,37	29,18	56,56	W-F	28,10	30,86	58,96
W-Dm[a]	26,31	29,91	56,22	W-Dm[a]	32,40	28,96	61,36
W-Dm	45,77	19,36	65,13	W-Dm	45,89	24,89	70,77
W-E	10,44	45,37	55,81	W-E	16,60	38,94	55,54
W-De	11,90	43,13	55,03	W-De	19,30	36,48	55,78
G-F	36,51	17,98	54,50	G-F	34,00	20,65	54,65
G-Dm[a]	28,09	25,29	53,38	G-Dm[a]	33,50	21,01	54,51
G-Dm	45,77	12,05	57,82	G-Dm	45,89	14,34	60,22
Dm-E	45,77	5,49	51,26	Dm-E	45,89	7,85	53,73
E-De	14,58	35,81	50,39	E-De	21,90	25,28	47,18
Average	*28,44*	*27,10*	*55,54*	*Average*	*31,34*	*25,84*	*57,18*
100 W 1 MINUTE PLASMA				**100 W 1 MINUTE PLASMA**			
W-G	13,26	45,10	58,36	W-G	17,90	40,75	58,65
W-F	21,99	36,36	58,35	W-F	25,70	34,80	60,50
W-De	11,77	46,97	58,73	W-De	20,10	38,76	58,86
G-F	34,33	18,96	53,29	G-F	33,00	20,74	53,74
G-De	10,64	50,67	61,31	G-De	11,70	62,81	74,51
E-De	25,94	17,86	43,80	E-De	27,30	16,61	43,91
Average	*19,65*	*35,99*	*55,64*	*Average*	*22,61*	*35,74*	*58,36*
300 W 1 MINUTE PLASMA				**300 W 1 MINUTE PLASMA**			
W-G	15,08	42,10	57,18	W-G	18,90	39,04	57,94
W-F	26,83	31,82	58,65	W-F	28,50	32,59	61,09
W-Dm[a]	23,91	34,00	57,91	W-Dm[a]	31,20	31,32	62,52
W-Dm	44,53	21,76	66,29	W-Dm	44,71	26,83	71,54
W-De	11,47	46,43	57,89	W-De	19,60	38,43	58,03
G-F	43,89	12,75	56,64	G-F	38,40	17,52	55,92
G-Dm[a]	26,67	26,26	52,94	G-Dm[a]	32,40	21,44	53,84
G-Dm	44,53	12,39	56,92	G-Dm	44,71	14,51	59,22
G-De	8,93	55,36	64,29	G-De	19,90	36,87	56,77
F-Dm[a]	21,99	40,25	62,23	F-Dm[a]	31,70	26,02	57,72
F-Dm	44,53	12,26	56,79	F-Dm	44,71	12,77	57,48
Average	*28,39*	*30,49*	*58,88*	*Average*	*32,25*	*27,03*	*59,28*

[a] Dm with polar value was used.
W = Water, G = Glycerol, F = Formamide, E = Ethyleneglycol, De = Diethylene Glycol, Dm = Diiodomethane.

On the other hand, upon increase of power, polar components, demonstrated a continuous increase up to 59.77 mJ/m^2 with Geometric mean and 45.94 mJ/m^2 with Harmonic mean approaches. This was expected because many functional and active groups were created on the surfaces upon exposure to oxygen plasma. When the total SFE is examined, again a continuous increase with power was obtained; SFE values increased to about 65 mJ/m^2 with both approaches.

Application of Acid-Base Approach

In the calculation of acidic and basic components of SFE, the contact angles of 3 liquids with known polarities are needed and it was not easy to find these triplets since most of the liquids tested completely wetted the surfaces, especially the ones

Table 4.
Dispersive and polar components of SFE for the samples treated for 15 min.

Geometric Mean				Harmonic Mean			
Liquid Pair	Dispersive Component (*mJ/m*^{2})	Polar Component (*mJ/m*^{2})	Total (*mJ/m*^{2})	Liquid Pair	Dispersive Component (*mJ/m*^{2})	Polar Component (*mJ/m*^{2})	Total (*mJ/m*^{2})
	CONTROL				**CONTROL**		
W-G	23,37	17,73	41,10	W-G	20,00	23,07	43,07
W-F	21,83	18,61	40,44	W-F	21,60	22,60	44,20
W-De	19,27	20,25	39,52	W-De	19,70	23,40	43,10
G-De	16,28	25,61	41,89	G-De	19,70	23,41	43,11
Average	*20,19*	*20,55*	*40,74*	*Average*	*20,25*	*23,12*	*43,37*
	20 W 15 MINUTE PLASMA				**20 W 15 MINUTE PLASMA**		
W-G	12,70	46,64	59,34	W-G	17,80	41,62	59,42
W-F	26,32	33,74	60,05	W-F	28,60	33,85	62,45
W-Dm[a]	24,50	35,12	59,62	W-Dm[a]	32,00	32,23	64,23
W-Dm	45,75	22,47	68,22	W-Dm	45,87	27,67	73,54
W-E	5,40	58,42	63,82	W-E	14,10	45,78	59,88
W-De	10,46	49,67	60,13	W-De	19,40	39,82	59,22
G-F	47,23	10,97	58,20	G-F	40,40	16,51	56,91
G-Dm[a]	28,39	24,56	52,95	G-Dm[a]	33,50	20,65	54,15
G-Dm	45,75	11,75	57,50	G-Dm	45,87	14,07	59,94
G-De	8,81	55,76	64,58	G-De	19,90	36,75	56,65
F-Dm[a]	23,28	39,00	62,28	F-Dm[a]	32,80	25,36	58,16
F-Dm	45,75	12,02	57,77	F-Dm	45,87	12,66	58,53
Dm-E[a]	37,19	8,88	46,07	Dm-E[a]	36,70	10,40	47,10
Dm-E	45,75	4,65	50,41	Dm-E	45,87	7,06	52,93
Dm-De[a]	43,05	3,21	46,26	Dm-De[a]	40,10	5,78	45,88
Dm-De	45,75	2,17	47,92	Dm-De	45,87	3,79	49,66
E-De	22,79	21,31	44,10	E-De	25,70	18,17	43,87
Average	*30,52*	*25,90*	*56,42*	*Average*	*33,55*	*23,07*	*56,62*
	100 W 15 MINUTE PLASMA				**100 W 15 MINUTE PLASMA**		
W-G	10,89	50,88	61,77	W-G	16,90	44,11	61,01
W-Dm[a]	25,22	36,08	61,30	W-Dm[a]	33,10	32,99	66,09
W-Dm	47,07	23,09	70,16	W-Dm	47,13	28,46	75,60
W-E	8,01	55,36	63,37	W-E	16,50	44,56	61,06
W-De	10,86	50,91	61,77	W-De	20,10	40,96	61,06
G-Dm[a]	30,17	23,02	53,20	G-Dm[a]	34,80	19,88	54,68
G-Dm	47,07	11,17	58,24	G-Dm	47,13	13,70	60,83
G-De	10,85	50,98	61,83	G-De	21,20	34,74	55,94
Dm-E[a]	35,77	12,59	48,35	Dm-E[a]	36,80	12,72	49,52
Dm-E	47,07	6,05	53,12	Dm-E	47,13	8,52	55,65
Dm-De[a]	44,13	3,42	47,55	Dm-De[a]	41,00	6,14	47,14
Dm-De	47,07	2,26	49,33	Dm-De	47,13	4,00	51,13
E-De	16,69	34,68	51,37	E-De	23,80	24,81	48,60
Average	*29,29*	*27,73*	*57,03*	*Average*	*33,29*	*24,28*	*57,56*
	300 W 15 MINUTE PLASMA				**300 W 15 MINUTE PLASMA**		
W-G	0,67	83,12	83,79	W-G	8,90	62,78	71,68
W-Dm[a]	39,86	39,86	79,72	W-Dm[a]	34,90	35,82	70,72
W-Dm	49,69	25,43	75,11	W-Dm	49,69	31,07	80,77
W-De	8,62	59,77	68,39	W-De	19,50	45,94	65,44
G-Dm[a]	39,42	10,91	50,32	G-Dm[a]	38,90	13,28	52,18
G-Dm	49,69	6,16	55,85	G-Dm	49,69	9,41	59,10
G-De	21,38	25,64	47,02	G-De	24,50	23,29	47,79
Dm-De[a]	51,02	1,18	52,20	Dm-De[a]	46,00	4,26	50,26
Dm-De	49,69	1,52	51,21	Dm-De	49,69	3,41	53,10
Average	*34,45*	*28,18*	*62,62*	*Average*	*35,75*	*24,47*	*61,23*

[a] Dm with a polar value was used.

W = Water, G = Glycerol, F = Formamide, E = Ethyleneglycol, De = Diethylene Glycol, Dm = Diiodomethane.

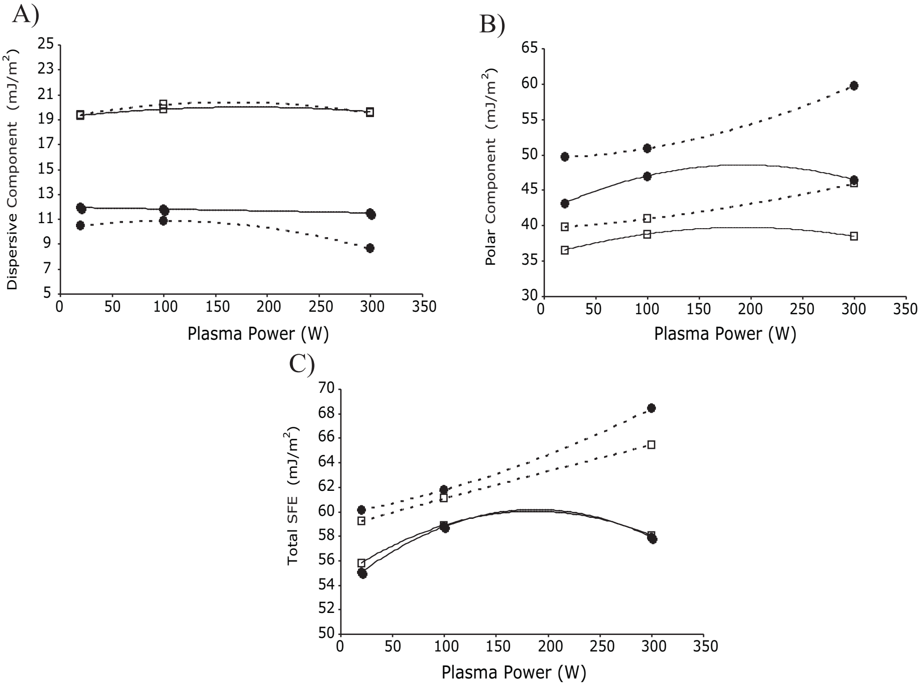

Figure 1.
Geometric and Harmonic Mean data for W-De pair versus applied plasma power. A) Dispersive components, B) Polar Components, C) Total SFE; ● Geometric mean, ☐ Harmonic mean, continuous lines = 1 min plasma, broken lines = 15 min plasma.

treated with 300 Watts plasma application. The results for SFE and its acidic and basic components obtained using the acid base approach are given in Table 5.

If the total SFE values are compared for the common triples of W-G-E, an increase from 36.27 mJ/m^2 to 45.03 mJ/m^2 is observed as the plasma power increased to 100 watts. The polar component values increased from 6.25 mJ/m^2 to 31.3 mJ/m^2, while the basic and acidic components increased from 9.20 mJ/m^2 to 29.31 and from 1.06 mJ/m^2 to 8.35 mJ/m^2, respectively. Dispersive components decreased from 30.02 mJ/m^2 to 13.73 mJ/m^2.

On the other hand, with the W-Dm-E and G-Dm-E triplets, dispersive components of all surfaces were found to be quite close to each other (about 45 mJ/m^2) with a slight increase with plasma power. Polar components were quite low; in the range of 1.06–4.19 mJ/m^2.

These very low polar component values are in contrast to the results obtained from the Geometric and Harmonic mean calculations. Considering that oxygen plasma increases the oxygen content, introduces new polar groups[11,12] and increases hydrophilicity, Geometric and Harmonic mean results seem more reliable. In the literature, different values are given for acidic and basic components of liquids. The values used in this study are the ones given by Della Volpe and Siboni,[8,9] It is possible to obtain different results if data from other sources is used. Acidic and basic components account for the hydrogen bonding and pi electrons[13] and the application of plasma causes random changes on the surfaces by forming new functional groups and active radicals, and these result, these changes might have caused large variations in the values of acidic and basic components. Therefore, for plasma treated surfaces, it is

Table 5.
SFE and its acidic and basic components obtained from the Acid-Base approach.

Liquid Triplets	γ_S (mJ/m^2)	γ_S^{LW} (γ_S^d) (mJ/m^2)	γ_S^{--} (mJ/m^2)	γ_S^{+} (mJ/m^2)	γ_S^{AB} (mJ/m^2)
CONTROL					
W-G-F	40,11	28,91	6,49	4,84	11,20
W-G-E	36,27	30,02	9,20	1,06	6,25
W-Dm-E	44,23	43,17	6,89	0,04	1,06
G-Dm-E	44,31	43,17	4,88	0,07	1,14
Average	*41,23*	*36,32*	*6,86*	*1,50*	*4,91*
20 W 1 MIN					
W-G-F	54,45	23,27	9,18	26,48	31,18
W-G-E	41,65	26,23	19,78	3.00	15,42
W-F-Dm	54,59	45,76	11,95	1,63	8,83
W-Dm-E	49,48	45,76	14,73	0,24	3,72
G-F-Dm	56,9	45,76	4,76	6,52	11,14
G-Dm-E	49,42	45,76	10,23	0,33	3,66
F-Dm-E	49,38	45,76	18,62	0,18	3,62
Average	*50,84*	*39,76*	*12,75*	*5,48*	*11,08*
100 W 1 MIN					
W-G-F	58,73	14,26	13,37	39,96	44,47
W-G-E	41,13	17,01	26,79	5,43	24,12
W-F-E	48,72	46,22	17,5	0,09	2,5
Average	*49,53*	*25,83*	*19,22*	*15,16*	*23,70*
300 W 1 MIN					
W-G-F	57,52	15,99	9,35	46,14	41,53
W-F-Dm	55,2	44,53	13,29	2,14	10,67
G-F-Dm	56,7	44,53	3,57	10,38	12,17
Average	*56,47*	*35,02*	*8,74*	*19,55*	*21,46*
20 W 15 MIN					
W-G-F	60,18	12,86	9,72	57,59	47,32
W-G-E	41,47	16,32	27,9	5,67	25,15
W-F-Dm	55,34	45,75	14,57	1,58	9,59
W-Dm-E	48,42	45,75	18,24	0,10	2,66
G-F-Dm	57,31	45,75	2,99	11,19	11,56
G-Dm-E	48,69	45,75	10,31	0,21	2,94
F-Dm-E	48,02	45,75	23,42	0,06	2,26
Average	*51,35*	*36,85*	*15,31*	*10,91*	*14,50*
100 W 15 MIN					
W-G-E	45,03	13,73	29,31	8,35	31,3
W-Dm-E	51,26	47,07	18,00	0,24	4,19
G-Dm-E	51,06	47,07	9,10	0,44	3,99
Average	*49,12*	*35,96*	*18,80*	*3,01*	*13,16*
300 W 15 MIN					
A-B-C	NR	NR	NR	NR	NR

NR indicates no result; A-B-C stands for all liquid triplets,
W = Water, G = Glycerol, F = Formamide, E = Ethyleneglycol, De = Diethylene Glycol, Dm = Diiodomethane.

advisable to consider only dispersive and polar components obtained from geometric or harmonic mean equations by using proper liquid pairs, instead of acidic and basic subcomponents.

Sample-Cell Interactions

MTS assays reveal that oxygen plasma application increased the cell attachment probably due to increased hydrophilicity (Table 6). The average number of cells

Table 6.
Variation of cell attachment with plasma power.

Plasma Power (Watts)	Average Cell Number
0	18,666
20	120,000
100	162,333
300	56,000
TCPS	276,333

attached on the untreated control group was about 19000 cells and this value increased up to 162000 for the modified surfaces with 100 W plasma applications, demonstrating a maximum at this point. When power was further increased to 300 W, this number decreased to 56000. This decrease may be explained by crosslinking or degradation of the polymer at higher plasma powers or by surface roughness changes. At 300 W more radicals than at lower powers form and this could lead to crosslinking on the surface. On the other hand increased hydrophilicity might have decreased the level of van der Walls interactions and thus decreased cell attachment. All the surfaces demonstrated less cell attachment compared to that on tissue culture polystyrene (TCPS) used as positive controls. Fluorescence micrographs of the cells attached to the surfaces are given in Figure 2.

The maximum cell attachment was obtained for the surfaces treated with 100 Watts for 15 min. and for the same surfaces the total SFE values calculated from Geometric and Harmonic mean approaches were found to be around 61 mJ/m^2. Therefore based on geometric and harmonic mean calculations, this value could be considered as the optimum for maximum cell attachment. In the previous studies based on

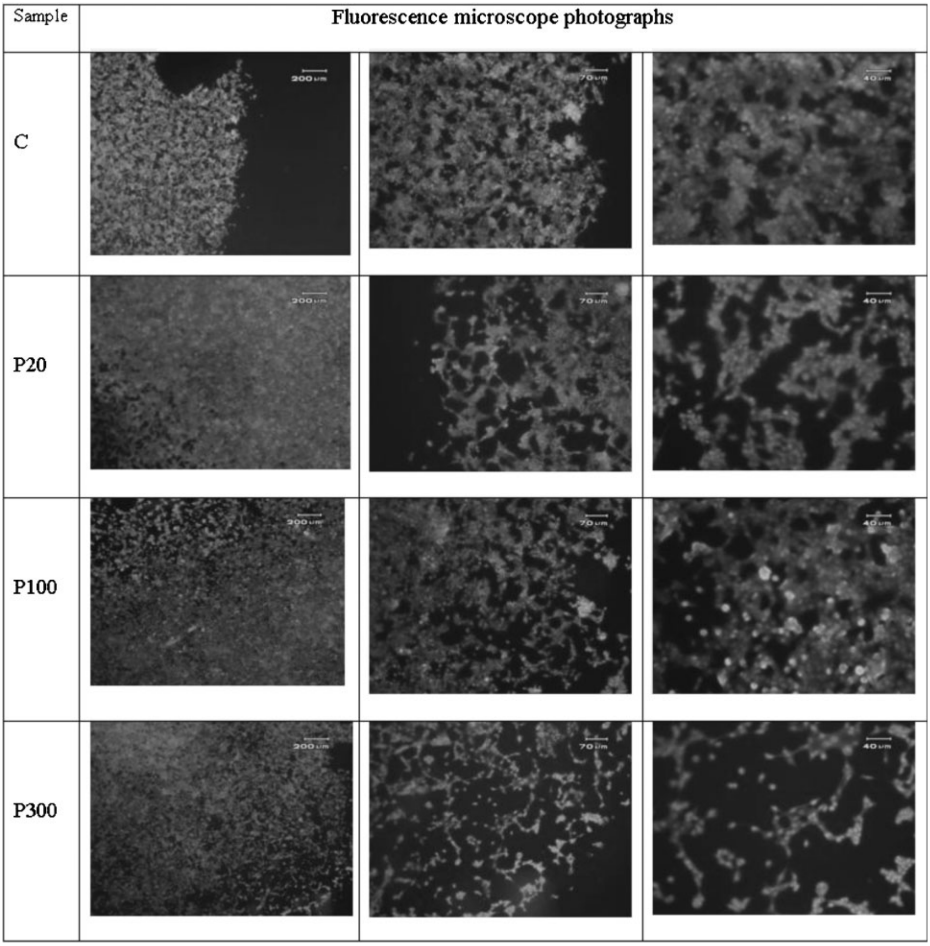

Figure 2.
3T3 cells attached cells on modified surfaces.

Zissman plot calculations, it was observed that total SFE of 40.34 mJ/m^2 demonstrated maximum cell attachment.[14] On the other hand, if acid-base approach is considered based on W-G-E triplet, the maximum cell attachment was obtained when the energies were about 45 mJ/m^2 for SFE, 14 mJ/m^2 for dispersive, 31 mJ/m^2 for polar, 8 for acidic and 29 mJ/m^2 for basic components. It should be considered that, the choice of liquid triplets significantly affect the results.

Conclusion

Plasma application is a good technique to modify surfaces without causing any substantial changes in the bulk properties of materials. In this study, PMMA films were modified by oxygen plasma application and an increase in hydrophilicities and surface free energies were observed as the power and application duration of plasma increased. Geometric and Harmonic mean equations were applied and the dispersive and polar components of SFE were calculated. Choice of liquid pair plays an important role and in this study most proportional change was observed for water-diethylene glycol pair.

In most of the literature, the results of SFE are given only for two or three liquid combinations.[15–20] Obtaining acidic and basic components just by one liquid triplet or obtaining polar and dispersive components just by one liquid pair might not be enough. The results obtained from acid-base approach were quite different from each other. Therefore, geometric and harmonic mean methods seem more appropriate due to the possibility that random changes of functionality might have been caused or liquid triplets were not compatible in the acid-base approach calculations.

The 100W plasma applied group demonstrated the highest cell attachment among all the samples. Both methods indicated a total SFE of around 61 mJ/m^2 and therefore, if there is a relation between SFE and cell attachment, this must be the value to be considered according to the Geometric and Harmonic mean equations.

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