Surface Modification of silicone based Materials for Voice Prosthesis

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Resumo

Este trabalho teve como objectivo a modificação da superfície de membranas de PDMS (polidimetilsiloxano) para aplicação em prótese da fala. Todos os materiais de base silicone para aplicação em prótese da fala sofrem da formação de um biofilme bacteriano, ao longo do tempo. No sentido de inibir ou minimizar a formação deste biofilme bacteriano foram utilizadas diversas técnicas comumente utilizadas na modificação de superfícies, tais como o enxerto de monómeros por radiação ultra-violeta (UV) e o enxerto de grupos amínicos. Também foi utilizada a técnica de modificação por plasma, com posterior enxerto de monómeros.

Numa fase inicial as superfícies modificadas foram analisadas através da determinação de ângulos de contacto com a água, pois estes permitem avaliar de uma forma expedita a modificação da superfície. Posteriormente as diferentes superfícies obtidas foram analisadas por Espectroscopia no infravermelho por transformada de Fourier e por microscopia electrónica de varrimento para investigação química da amostra e pelo método de Owens, Wendt, Rabel and Kaelble para determinação da energia livre de superfície. Por goniometria dos ângulos de contacto foi possível estimar a hidrofobicidade/hidrofilicidade da superfície ao longo do tempo e com a balança a diminuição/aumento do peso do material. Finalmente o estudo da citotoxicidade e da adesão celular à superfície dos materiais foi avaliada *in vitro*.

Ao longo deste trabalho foram utilizadas técnicas de modificação de superfícies como o enxerto de monómeros à superfície da membrana de silicone, recorrendo à radiação ultravioleta (UV) e ao plasma com Oxigénio e Argon. Os monómeros escolhidos para o efeito foram o metacrilato de hidroxietilo e o ácido metacrílico, pois reúnem características tais como biocompatibilidade, presença de grupos muito hidrofílicos e propriedades antibacterianas. Com estes métodos pretende-se que a introdução dos grupos hidrofílicos proveniente dos monómeros proporcionasse um aumento da componente polar e consequentemente uma diminuição no ângulo de contacto, o que permitiria uma maior resistência bacteriana. Na modificação por plasma, que consistiu na modificação da superfície utilizando como gases o oxigénio e o árgon, foram avaliados os diversos parâmetros (pressão e temperatura) para ambos os gases, de maneira a determinar as condições de processamento óptimas. No

entanto, esta técnica apresenta como debilidade o facto do PDMS não se manter estável ao longo do tempo, levando ao aumento da sua hidrofobicidade ao longo do tempo. Contudo, o enxerto de monómeros por plasma permitiu o aumento da estabilidade da hidrofilicidade da superfície ao longo do tempo. Esta técnica revelou-se eficiente pois permitiu a introdução grupos hidrofílicos na superfície, o que se traduziu no aumento da componente polar e na diminuição do ângulo de contacto.

Foram ainda enxertados grupos aminicos ($–NH_2$) na superfície da membrana de silicone recorrendo à 1,6 - hexanodiamina. Para tal foram averiguados 2 protocolos, contudo apenas um se mostrou eficiente na diminuição da hidrofobicidade. A caracterização por espectroscopia de infra-vermelhos evidenciou a presença de grupos amina. O sucesso na introdução de grupos amina traduziu-se num aumento da componente polar e consequentemente na diminuição do ângulo de contacto.

Todas as modificações de superfície a que os filmes de PDMS foram sujeitos foram avaliados ao longo de um mês em diferentes meios e verificou-se que a ocorreu recuperação da hidrofobicidade na técnica de enxerto de monómeros por radiação ultra-violeta (UV), enxerto de grupos amínicos e na modificação por plasma, o que sugere reorientação das cadeias poliméricas. A percentagem de inchaço foi praticamente nula em todas as técnicas, excepto na modificação com a amina, que se pode dever à interacção dos grupos amina com o meio.

Por último, foi realizada a caracterização da citotoxicidade e da actividade antibacteriana das membranas de PDMS. Nos testes de caracterização da citoxicidade nenhuma das amostras afectou a integridade ou viabilidade celular, o que é fundamental para a sua utilização em aplicações biomédicas. Nos ensaios de caraterização da actividade antibacteriana das amostras verificou-se que as amostras possuem uma baixa actividade antibacteriana. Os resultados obtidos revelaram uma redução do crescimento bacteriano nas amostras em que houve enxerto de MAA por plasma e enxerto de HEMA por UV.

Abstract

The main purpose of this research work was the modification of PDMS (polydimethylsiloxane) films for application in voice prosthesis. All silicone based materials for voice prosthesis suffer from microbial biofilm formation, along time. In order to inhibit or minimize its microbial biofilm formation different surface modification techniques were used, such as: UV (ultra-violet) grafting, chemical grafting of amino groups and plasma activation for surface modification with subsequent grafting of monomers.

In an initial phase, the characterization of the modified surfaces was accomplished by determining water contact angles, because this technique allows to evaluate the surface modification, in an expedite way. The water contact angle allows to estimate the hydrophobicity / hydrophilicity of the surface over time and to balance the decrease / increase of the weight of material before and after the modification. Afterwards, the different obtained surfaces were analyzed by Fourier transform Infrared Spectroscopy (FTIR) and scanning electron microscopy (SEM) for chemical evaluation and determination of the surface free energy by the Owens Wendt, Rabel and Kaelble method. Finally, the study of the cytotoxicity and cell adhesion to the surface of the materials was evaluated *in vitro*.

Throughout this work the techniques used for surface modification by grafting monomers onto the surface of the PDMS films were ultraviolet (UV) irradiation and plasma surface activation with oxygen and argon. The chosen monomers for the grafting were hydroxyethyl methacrylate (HEMA) and methacrylic acid (MAA), due to characteristics such as: biocompatibility, the presence of hydrophilic groups and antibacterial properties. By using these modification methods is intended to introduce hydrophilic groups from monomers in the surface, which would provide an increase of the polar component, and consequently a decrease in water contact angle. This water contact decrease allows an increase of the bacterial resistance. In the modification by plasma, which consisted of surface activation using oxygen and argon as working gases, several parameters were evaluated (pressure and temperature) for both gases, to determine optimal activation conditions. However, this technique showed as a weakness the fact that the PDMS does not remain stable along time, leading to an

increase of hydrophobicity along time. However, by grafting a monomer to the surface after the plasma activation the increase of the hydrophilicity stability of the surface along time could be achieved. This technique has proved to be efficient because it allowed the introduction of hydrophilic groups at the surface.

Also, to the surface of PDMS films were grafted amine groups (-NH_2) by using 1.6 - hexanediamine. For such propose, two protocols were explored, but only one proved to be effective in the decrease of the hydrophobicity. The characterization by infrared spectroscopy showed the presence of amine groups and the successful introduction of these groups resulted in an increase in the polar component (measured by surface free energy, using the OWRK method)) and consequently in the decrease of the water contact angle. All surface modifications of PDMS films were evaluated over a month in different storage mediums and it was found that the hydrophobicity recovery occurred in the ultraviolet radiation (UV) grafting, grafting of amine groups and modification by plasma, which suggests reorientation of the polymer chains. The percentage of swelling was practically null in all techniques except in the modification with amine, which may be due to the interaction of the amine groups with the medium.

Finally, the cytotoxicity of all the modified films was evaluated and the antibacterial activity of the PDMS films determined. The cytotoxicity tests showed that none of the films affected cell integrity or viability which is fundamental for the biomedical application proposed for this material. The antibacterial activity assays revealed that samples with MAA grafted by plasma activation and films grafted with HEMA by UV a reduction of the bacterial growth was induced.

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Acronyms

HEMA – Hydroxyethylmethacrylate

MAA – Methacrylic acid

PDMS – Polydimethylsiloxane

FTIR - Fourier Transform Infrared Spectroscopy

SEM - Scanning Electronic Microscopy

UV – Ultraviolet

OWRK – The Owens, Wendt, Rabel and Kaelble method used on the determination of the free surface energy

LMWS - Low molecular weight species

PBS - Phosphate buffered saline

(PDMS+HEMA)_growth medium – PDMS with HEMA, under growth medium

(PDMS+MAA)_growth medium – PDMS with MAA, under growth medium

(PDMS+HEMA)_distilled water – PDMS with HEMA, under distilled water

(PDMS+MAA)_distilled water – PDMS with MAA, under distilled water

(PDMS+HEMA)_PBS – PDMS with HEMA, under PBS

(PDMS+MAA)_PBS – PDMS with MAAA, under PBS

MTS – 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium

FBS – Fetal bovine serum

DMEM-F12 – Dulbecco's modified Eagle's medium

EDTA – Ethylenediaminetetraacetic acid

LB – Lysogeny broth

Motivation

The main motivation of this research work was the need of an answer to a current problem in implants: the formation of a biofilm on the surface of medical devices, in this particular case, voice prosthesis. The formation of this biofilm is the main cause of malfunction, or ultimately of the replacement of the currently used voice prosthesis. The surface modification of materials is one of the most used and studied approaches in recent years, in order to get an answer to this problem. Therefore, in order to reduce adhesion of microorganisms, the surface modification of silicone based materials would be sought for their application in voice prosthesis.

Thus, several techniques have been developed and refined towards the surface modification of a silicone based materials – Sylgard 184®. In this research work, the surface modification techniques used were UV (ultra-violet) grafting, chemical grafting of amino groups and plasma activation for surface modification with subsequent grafting of vinyl monomers – hydroxyethyl methacrylate (HEMA) and methacrylic acid (MAA). Comparatively to other modification techniques (such as corona discharge, laser treatments, gamma-ray, electron beam, ion beam and the use of biosurfactants) these modifications techniques present some advantages such as: low cost of treatment, fast reaction rate, simple equipment and industrialization and improvement of the surface properties of a material without affecting its bulk properties.

1. Introduction

1.1 Voice prosthesis

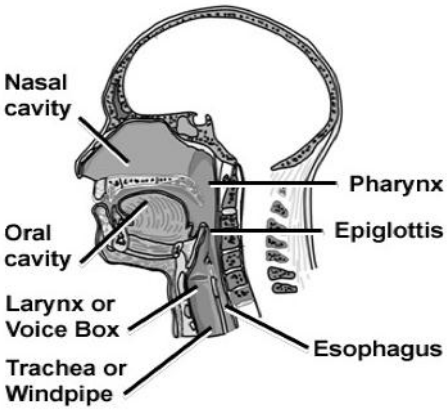

Figure 1.1 - Upper respiratory system [130]

In the upper and front part of the neck is located the larynx. Its superior edge is located below the pharynx and the root of the tongue and its inferior margin has a membranous connection with the upper ring of the trachea. The larynx has two main and important functions: it changes our physical condition (the admission of air to the lungs, some degree of regulation in its quantity, and conferred resistance to the entry of foreign bodies) and is responsible for the generation of the voice. [1] In figure 1.1, can be observed the position of the larynx, in a human body (upper respiration system).

One of the basic human attributes is the voice [2]. Through a simple mechanical setup the voice phenomena is produced. The larynx, which is placed on top of the trachea, helps to send air into the lungs during expiration. Thus, the air delivered creates vibrations in specific elastic and tense membranes, the boundaries of a chink (that is the orifice of entrance and of exit for the supply of air to the lungs). These vibrations generate voice. [3]

Therefore, is not surprising that one of the most dangerous effects of a total laryngectomy (surgical treatment due to extensive cancer of larynx) is the loss of voice. [2], [4] Disfigurement and a large part of laryngeal functions (control of airways, phonation, swallowing effort closure during strenuous activity and cough) will be seriously affected after surgical removal of the larynx as malignancies laryngopharyngeal primary treatment or as rescue treatment after recurrent cancer. [2]

There are two types of voice prosthesis, the indwelling devices, which keep in the stand for an extending period of time, such as the Groningen button, Traissac et

al., Nijdam, Provox and Staffieri and removable devices, which for cleaning reasons, must be frequently removed, such as the Blom, Singer and Panje.

Presently, the most used devices in Europe are the self-retaining low resistance Provox voice prosthesis developed in the Netherlands Cancer Institute in 1988 along with the Groningen button voice prosthesis. [4]

Many patients who had a laryngectomy show problems concern to the blockage of the valve, which can cause discomfort, coughing and pneumonia. The frequent replacement of the valve has adverse effects in the life quality of the patients. [5]

The initial implant of a voice prosthesis (originally invented and implemented by Mozolewski in 1972) was performed by Blom and Singer. This device became an international instrument available in 1980. [6]

Because of the increased airflow resistance or retrograde leakage of fluid into the trachea due to biofilm formation, tracheoesophageal voice prostheses have to be substituted several years after being implanted. [7]

There are two types of obstruction: through the valve or around the valve. The first type of obstruction is caused by dysfunction of the valve, particularly due to biofilm formation. The second obstruction is determined by the size of the valve wall and the fistula. [5]

1.2 Silicone: General description and history

For future biomedical applications, PDMS (polydimethylsiloxane) systems have been studied. [8] These materials offer great properties that will be discussed, in detail, in the next chapter.

Table 1.1 resumes the key milestones in the advances of silicone chemistry.

Table 1.1 - Key milestones in the advancement of silicone [9], [10], [11]

1824 Berzelius discovers silicon by the reduction of potassium fluorosilicate with potassium 4K+K2SiF6→Si + 6KF. Reacting silicon with chlorine gives a volatile compound later identified as tetrachlorosilane, SiCl4Si+2Cl2 → SiCl4.
1863 Friedel and Craft synthesize the first silicon organic compound, tetraethylsilane 2Zn (C2H5)2+ SiCl4 → Si (C2H5)4 + 2ZnCl2.
1871 Ladenburg observes that diethyldiethoxysilane, (C2H3)2Si (OC2H5)2, in the presence of a diluted acid gives an oil that decomposes only at “a very high temperature”.
1901-1930 kipping lays the foundation of organosilicon chemistry with the preparation of various silanes by means Grignard reactions and the hydrolysis of chlorosilanes to yield “large molecules”. The polymeric nature of the silicones is confirmed by the work of Stock.
1940s silicones become commercial materials after Hyde of Dow Corning demonstrates the thermal stability and high electrical resistance of silicone resins, and Rochow of General Electric finds a direct method to prepare silicones form silicon and methylchloride.
1989 The use of silicones in pharmaceutical and biomedical applications was around 14,000 tons, and the amount of silicon implanted, in the long term, was approximated 90 tons
1999 The annual consumption of silicone elastomers grew by 1.7 million tons.

1.2.1 Silicone structure

In 1901, by similarity with ketones, Kipping gave the name “silicone”, to designate new compounds of the brut formula R_2SiO. These were instantly identified as being a polymer and analogous to polydialkylsiloxanes, with the formulation shown in figure 1.2: [12]

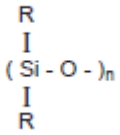

Figure 1.2 - Compounds with formula R_2SiO [12]

The name of silicone was called and adopted by the industry and normally refers to linear polymers where R=Me, or polydimethylsiloxane (PDMS). In figure 1.3, can be observed the chemical structure of PDMS.

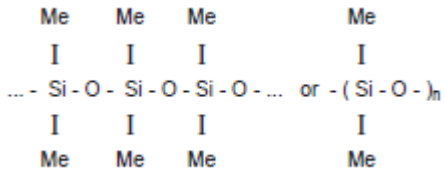

Figure 1.3 - Chemical structure of polydimethylsiloxane [12]

Normally, the side groups are methyl, however they can also be phenyl for severe low temperature performance or tri-fluoro propyl for upgraded oil and fuel resistance. Silicone elastomers have an inorganic oxygen and silicone backbone (extremely resistive to weathering).

Silicones have a combination of distinctive properties due to the simultaneous presence of “organic” groups connected to an “inorganic” backbone, and enable their use in a lot of fields such as aerospace (low and high temperature capacity), electronics (electrical insulation), in the building industries (resistance to weathering) or health care (great biocompatibility). [13]

PDMS materials have a large variety of applications and their structural alterations might be attributed to the next factors:

- Chemical stability (except on rough alkaline conditions), which explains the innocuous behavior of PDMS materials concerning to living tissues;
- Thermal stability of PDMS materials in comparison with similar carbon structures, which results of the relatively high Si-O-Si bond energies; for long periods of time, silicone elastomers stay flexible as low as -80ºC and constant at temperatures as high as 300ºC. Virtually, they don’t change after extended weathering and their

tremendous electrical proprieties still stable with frequency and temperature. [14], [15]

- Small rotational and bending energies confer flexibility to O-Si-O-Si bonds, as compared to similar carbon structures, because of the relatively large silicon bond radius; PDMS owns a distinctive flexibility (the shear modulus G could fluctuate between 100kPa and 3MPa). Siloxane chains might adopt a lot of configurations and their barriers to rotations are low. [16], [17]
- The capacity of silicon to expand his outermost electron shells beyond the octet;
- The exposition of methyl groups makes the surface very hydrophobic. Normally, the siloxane chain follows a configuration such, in which the chain exhibits a maximum number of methyl groups to the outside, while in hydrocarbon polymers the relative stiffness of the polymer backbone doesn´t allows a "selectively" exposure of the hydrophobic and methyl groups. [16]

Polydimethylsiloxanes have a low surface tension (20,4 mN/m) and are able of wetting most surfaces. Its surface has good release proprieties, especially if the film is cured after application, by the fact that methyl groups are pointed outward. By the fact that silicones are able of wetting themselves, it favors good film formation and good surface coverage. Their critical surface tension of wetting (24mN/m) is greater than its own surface tension. The viscous movement activation energy is highly low for silicones. In comparison to hydrocarbon polymers, their viscosity is less dependent on temperature. [18]

Besides of the proprieties referred above, PDMS has a low glass transition temperature (Tg ≈ -125ºC), high dielectric strength (~21kV/mm), high gas permeability, high compressibility, low chemical reactivity (except at extremes of pH), non-toxic nature, low cost and optical transparency. [17], [19], [20]

1.2.2 Silicone as a biomaterial

In 1982, was given the first possible definition of a biomaterial, by professors Dee, Puleo and Bizios: “Any substance (other than a drug) or combination of substances, synthetic or natural in origin, which can be used for any period of time, as a whole or part of a system, which treats, augments, or replaces any tissue, organ, or function of the body”. In 1987, was added an agreement definition of a biomaterial, often referenced in the literature, which could be assigned by the teacher David F.Williams:” A biomaterial is a nonviable material used in the production of a medical

device, intended to interact with biological systems". However, these two definitions referred before were not totally perfect or complete, because development of materials containing living cells, like artificial organs, defies the word nonviable. [21]

In 1992, the term nonviable was eliminated and a biomaterial agreement definition was setting: "A material intended to interface with biological systems to evaluate, treat, augment, or replace any tissue, organ, or function in the body". [21], [22]

Usually, biomaterials studies concentrate on problems such as biocompatibility, host-tissue reactions to implants, cytotoxicity and basic structure-propriety associations. These issues are essential, because they give a strong scientific basis to the understanding of medical devices such as voice prosthesis. But, as a primary worry, in biomaterials engineering, the manufacturing and processing aspects appear. There are, normally, four properties that biomaterials must have: biocompatibility, sterilizability, functionability and manufacturability. It´s very important to establish a production of a thousand units of identical devices that ensures good quality control, reliable proprieties and having to be packed in a sterile mode for simple transportation and storage. Durability, surface modification and corrosion are the major elements in engineering biomaterials for medical applications. [23]

From a clinical point of view a material to be compatible cannot cause toxic reactions, allergic, inflammatory or thrombogenic effects in the body, cannot cause deterioration of adjacent tissues or lead to carcinogenic effects, among other. [24]

During the past three decades, the PDMS elastomers has also been used as biomaterials in medical devices, emphasizing among others, artificial hearts, heart valves, breast implants, devices for ophthalmology (ocular lenses, implants for glaucoma), nose, artificial ears and skin, biosensors, catheters and prosthetic speech. Many biomedical applications take advantage of the properties offered by silicone elastomers. Among the silicone applications are included the systems of drug release, surgical specialties, tunnel for metal implants, orthopedic implants, and also particular materials such as pipes and valves. [25]

Numerous problems have been arisen when the implants are implanted for a long period of time, even though the silicone shows excellent properties of bioinerticity, stability and smoothness. Because of its hydrophobicity, the PDMS materials cannot be used in many applications. This occurs because the body recognizes silicone as invaders hydrophobic (foreign) materials by stimulating inflammation and fibrous capsules that isolate the biomaterial. This capsule affects the proper functioning of the implant, producing a physical barrier between the implant and the surrounding tissue causing contraction of the material. [26]

1.3 Sylgard 184®

Sylgard 184®, a silicone elastomer Kit, will be used as the base material in this work.[27] The main features of this compound are:

- High Transparency which allows simple Inspection of components.
- Fast and versatile cure processing controlled by temperature.
- Could be considered for uses requiring Underwriters Laboratories (UL) and Mil Spec (military specifications) requirements.
- High Tensile Strength.
- Flowable.
- No solvents or cure byproducts.

Sylgard 184 could be used for protection of electronic/electrical devices, as well as for some sealing applications (power supplies, high voltage resistor packs connectors, sensors, adhesive/encapsulant for solar cells, industrial controls, transformers and amplifiers). [27]

Sylgard®184 consists of a base (part A) and a curing agent (part B). The base consists of dimethylsiloxane oligomers with terminal vinyl groups and a platinum catalyst. The curing agent consists of dimethyl hydrogen siloxane groups. As a result of the curing reaction is obtained the polydimethylsiloxane. The reaction is schematized in figure 1.4. [27]

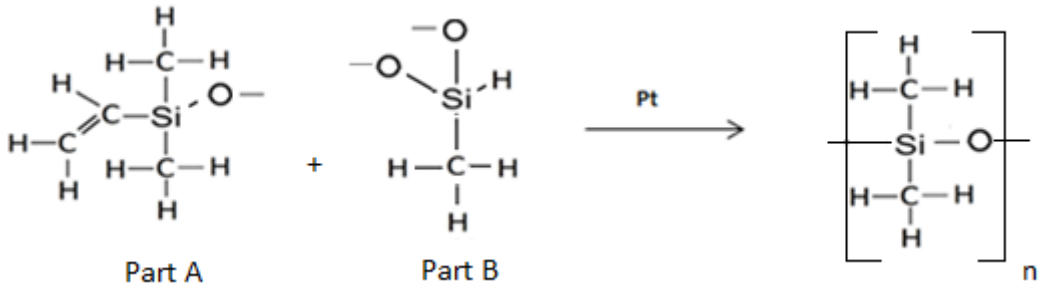

Figure 1.4 - Representation of the formation of the PDMS [27]

However, the data published about this product is limited. [28]

1.4 Biofilm Formation

Highly structured communities of microorganisms are defined as biofilms, which are surface-associated and/or closed to one another, enclosed inside a self-produced protective extracellular matrix. These might be formed in the natural environment, but also inside the human host, cooperatively interacting in an altruistic manner as complex cities. When an organism forms a biofilm, it gets some advantages like resistance to physical and chemical removal of cells, metabolic support, protection from the environment and a community-based regulation of gene expression. [29]

Bacteria are found mainly in biofilms, in most natural situations. The general recognition that biofilms have impact in many environments, from water pipes to indwelling devices in hospital patients, brought a rising interest in investigating the molecular systems underlying maintenance and formation of these communities. [30]

In some environments, bacteria might adhere to the majority of surfaces, via cell surface structures like *pili, fiambriae* and extracellular polymers or just by physicochemical interaction forces. The adhesion phenomena by physicochemical basis is the equilibrium between electrostatic and Van der wall´s forces as hydrophobic surface interactions, which are created in either repulsion or attraction between particles. Hydrophobic surface interactions are very attractive and allow adhesion of microorganisms to epithelial cells and abiotic surfaces. [31]

The formation of biofilm occurs in distinct steps, as shown in figure 1.5: microbial attachment, microbial proliferation and the subsequent formation of a bacteria biofilm. [31]

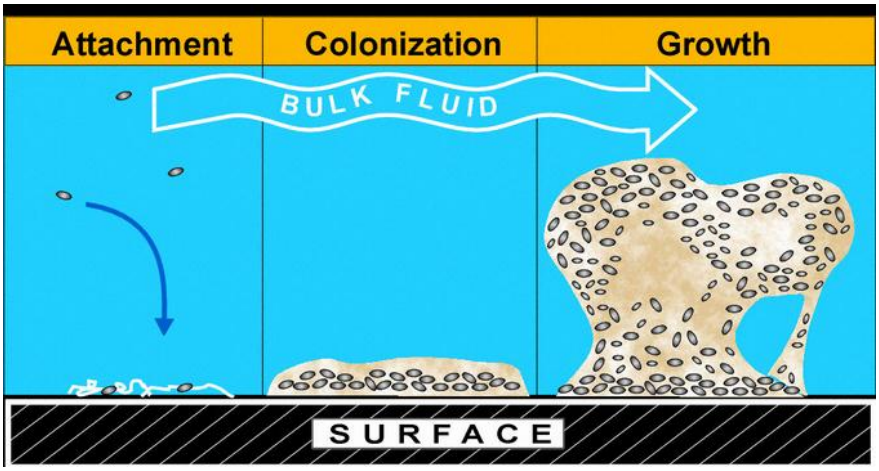

Figure 1.5 - Sequential stages in the formation of a biofilm

1.4.1 Silicone Biofilm

Failure of a device is attributed to the formation of the biofilms on medical devices. In laryngectomized patients, after some use, the voice prosthesis suffers deterioration and degradation (inhibiting its correct functioning) and should be replaced after three/ four months. [32]

Voice prostheses are very vulnerable to be colonized by microorganisms, mostly by *Candida spp*., growing in biofilms on the surface, which causes faulty of the valve (improper closure), an augment in air flow resistance and probably fluid leakage from the esophagus into the trachea. Therefore, it is necessary surgical replacement of the voice prosthesis. [33], [32]

The microbial colonization on voice prosthesis cannot be prevented. The velocity of colonization and the composition of biofilm depend on the characteristics of the material with which prosthesis is produced, the formation of physiological flora of an individual, and on a series of exterior agents like temperature, nutritional, humidity and other agents that are mainly the results of patient's routine. [34]

1.4.2 Factors that influence microbial adhesion

Before testing materials for bacterial adhesion, a complete knowledge of the chemical and physical properties of the materials is needed. The factors that could influence microbial adhesion are: roughness and topography, environment, surface chemistry and surface free energy. [35], [36], [37]

1.4.2.1 Roughness and topography

Atomic force microscopy (AFM) or profilometry are typically used to measure surface roughness. Some of the roughness parameters presented for assessing biomaterials are summarize on table 1.2. In bacterial adhesion, topography is an essential factor. For instance, the interaction of bacteria with two surfaces of equal chemistry, but contrary topography might result in considerably different densities of adherent bacteria *in vitro*. Roughening a surface creates turbulent fluid flow and augments the available surface for colonization. Consequently, increasing topography might lead to increased bacterial adhesion. [38]

Table 1.2 - Some of the roughness parameters available for characterizing a surface [38]

Roughness Parameter	Abbreviation	Description
Roughness Average	**Ra**	**Measures the average height of the surface**
Root mean Squared roughness	**Rq/RMS**	**Measures the average deviation of the surface from the mean height**
Skew	**Rsq**	**A measure of whether the surface is primarily composed of valleys of peaks**
Kurtosis	**Rk**	**Describes whether the surface is spiky(Rk>3), bumpy (Rk<3), or random (Rk=3)**

1.4.2.2 Environment

Time of exposure, bacterial concentration, temperature, associated flow conditions and the presence of antibiotics are factors that influence bacterial adhesion. [39]

1.4.2.3 Surface chemistry

A meticulous cross reference of five different termination groups is showed in table 1.3, submitted by Van der Vegte and Hadziioannou. It was discovered that different pair of van der Waals interacting tips revealed the weakest adhesive forces. But, their data demonstrated that by switching the termination groups on the tip and substrate they did not constantly see the identical adhesive force. [40]

Table 1.3 - Single Chemical Bond Forces (in pN) for every Tip-Substrate combination [40]

	Substrate				
Tip	**CH3**	**OH**	**NH2**	**COOH**	**HCONH2**
CH3	**81**	**57**	**59**	**61**	**601**
OH	**50**	**101**	**113**	**112**	**117**
NH2	**54**	**88**	**98**	**95**	**100**
COOH	**95**	**109**	**105**	**114**	**137**
CoNH2	**62**	**110**	**102**	**125**	**120**

Bacterial adhesion and proliferation are influenced by surface chemistry. Depending on material charge and hydrophobicity, materials with various functional groups change bacterial adhesion. [39]

In aqueous suspension, bacteria are practically always negatively charged. According to bacterial species, the surface charge of bacteria can change and is determined by the pH, the growth medium and the ionic strength of the suspending buffer, bacterial surface structure and bacterial age. Nevertheless, the contribution of bacterial surface charge to bacterial adhesion has not been totally understood. [39]

Bacteria adhere in a different way to materials with different hydrophobicities. Hydrophobic materials are less resistant to bacterial adhesion than hydrophilic materials. [41]

1.4.2.4 Surface free energy

In 1979, Baier and Dexter were among the first investigators to establish a correlation between adhesion of fouling organisms with the surface free energy of the substratum. The relative amount of bioadhesion and the surface energy is correlated in figure 1.6, which is known as the "Baier Curve". The main feature of this curve is that the minimum in the relative adhesion, at 22-24 $nM.m^{-1}$, (mJ/m^2), doesn´t happen at the lowest surface energy. [35]

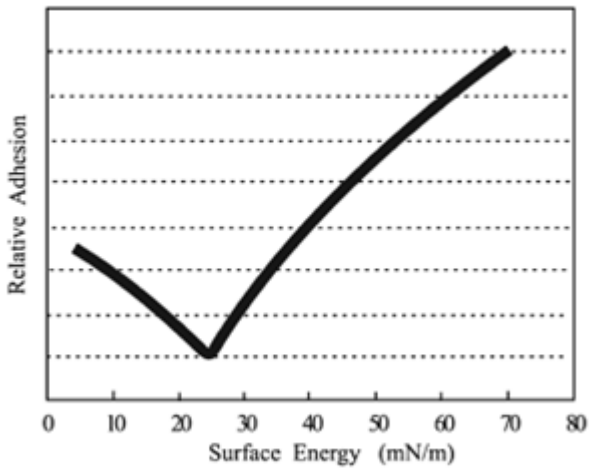

Figure 1.6 - The "Baier" curve [35]

In 2004, Zhao et al. studied the effect of surface free energy on bacterial adhesion and announced the optimum surface free energy, where the bacterial adhesion force is minimal, to be approximately 20-30 $nM.m^{-1}$. [35]

In 2006, Meyer et al, established that silicone coatings with critical surface tension between 20 and 30 $nM.m^{-1}$, release more easily different types of biofouling than materials of higher or lower critical surface tension. It was also demonstrated that some contact angle irregularities indicate that surface-active obtains from silicone coating inhibit the adhesive systems of fouling organisms. [35]

The control of the substratum surface free energy for capability of adhesion and binding strength has a general value like for (i) the colonization of vascular prosthesis for abdominal wall reconstruction, (ii) the adhesion of uropathogens to

polymer materials, (iii) the adhesion of catheter-associated bacteria, (iv) the binding strength of green alga to some surfaces, (v) the adhesion of *Salmonella typhimurium* to soil particles, (vi) the attachment of insect residues to aircraft wings and (vii) the attachment of freshwater bacteria to solid surfaces. [37]

1.5 The monomers

In this work, PDMS was grafted with three monomers: the HEMA (2-hydroxyethyl methacrylate), the MAA (Methacrylic acid) and the 1.6-hexanediamine.

For several years, the use of coating agents in combination with peroxides to cure rubbers has been a regular practice in the rubber industry. Usually, coagents are multifunctional monomers that are highly reactive in the presence of free radicals and readily graft to rubber chains to constitute a polymeric crosslink network. Methacrylic acids behave as coagents in the same mode as the methacrylate esters do (figure 1.7). [42]

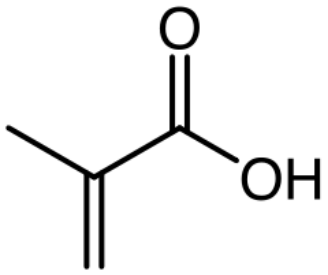

Figure 1.7 - Chemical structure of Methacrylic acid [90]

Because of the biocompatibility and the antibacterial properties, acrylics acids are commonly used as adhesives and superabsorbents materials due to its pendant carboxylic groups. Polymers grafted by acrylic acids develop highly hydrophilic materials and attractive matrixes for biomedical applications [43]

HEMA (2-hydroxyethyl methacrylate) (figure 1.8) is a biocompatible water absorbing plastic used to create ophthalmic prostheses (contact or intraocular lenses),

vascular prostheses, drug delivery devices and soft-tissue replacements. Is obtained by the reaction of methacrylic acid with ethylene oxide or propylene oxide: [44], [45]

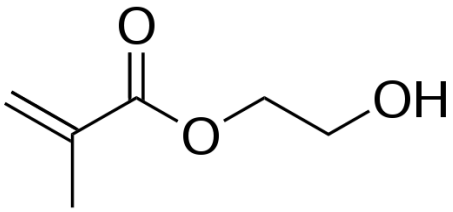

Figure 1.8 - Chemical structure of 2-hydroxyethyl methacrylate [46]

1.6 - Hexanediamine (figure 1.9) is an organic compound with the formula $H_2N(CH_2)_6NH_2$. This diamine molecule consists of a hexamethylene hydrocarbon chain terminated with amine functional groups. Because of the electronegativity of the nitrogen atom, C-N and N-H bonds have polarity, with the partial negative charge located on the nitrogen. Therefore, most amine compounds have a dipole which promotes aqueous solubility via dipole-dipole interactions with water molecules. [46], [47]

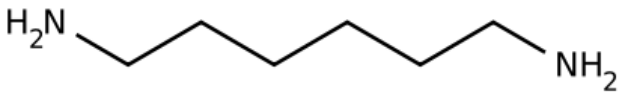

Figure 1.9 - Chemical structure of 1.6-Hexanediamine [46]

1.6 Surface modification – an introduction

The main reason for the surface modification of biomaterials is simple: to keep the fundamental physical properties by changing only the outermost surface in order to control biointeraction. If the surface modification is properly made, the bulk mechanical properties and features of the medical device are not changed, while the biological performance is improved. [48]

Surface modification gives flexible ways for improving surface properties such as: hydrophilicity, biocompatibility, anti-fouling, surface roughness, antistatic and

antibacterial properties, and even conductivity, while conserving the bulk structure of the base material. [49]

The main methods for surface modification can be grouped in two types: the chemical surface modification and the physical surface modification methods. The latter ones include flame, corona discharge, laser treatments, gamma-ray, electron beam, ion beam, plasma, and UV. [50] The principle operation of the last two techniques will be described, in more detail, in the next chapters.

Corona discharge is a well-recognized, relatively easy and one of the most commonly used continuous process for the surface modification. It consists of a high voltage-high frequency generator, an electrode and a grounded metal roll covered with an insulating material. In this method, when a high voltage is applied across the electrodes it ionizes the air generating plasma, also known as corona discharge. As a result of corona discharge physical and chemical changes happen on the polymer surface for improved adhesion. [51]

The use of surface active molecules (Biosurfactants) has become a significant product for industrial and medical applications. Biosurfactants are surface-active compounds which are produced by microorganisms like glycolipids, lipopeptides, polysaccharide–protein complexes, phospholipids, fatty acids, and neutral lipids. Biosurfactants present various advantages such as low toxicity, biodegradability, chemical variety, efficiency under extreme environmental conditions, surface activity, emulsifying capacity, antimicrobial and antiadhesive properties. However, these compounds present some disadvantages. The amounts of produced Biosurfactants are very low and the mechanism of microbial adhesion inhibition is little known. [4], [19]

To modify the chemistry of a surface, there are hundreds of chemical reactions that could be used. Chemical reactions can be grouped as nonspecific and specific. Nonspecific reactions leave a division of several functional groups at the surface. An example of a nonspecific reaction is the chromic acid oxidation of polyethylene surfaces. Other examples are radio-frequency glow discharge (RFGD) processing of materials in argon, nitrogen, oxygen or water vapor plasmas; corona discharge modification of materials in air and the oxidation of metal surfaces to a mixture of suboxides. Specific reactions modify only one functional group into another with a high field and a small amount of side reactions. [52]. In figure 1.10, examples of specific chemical surface modifications for polymers are illustrated.

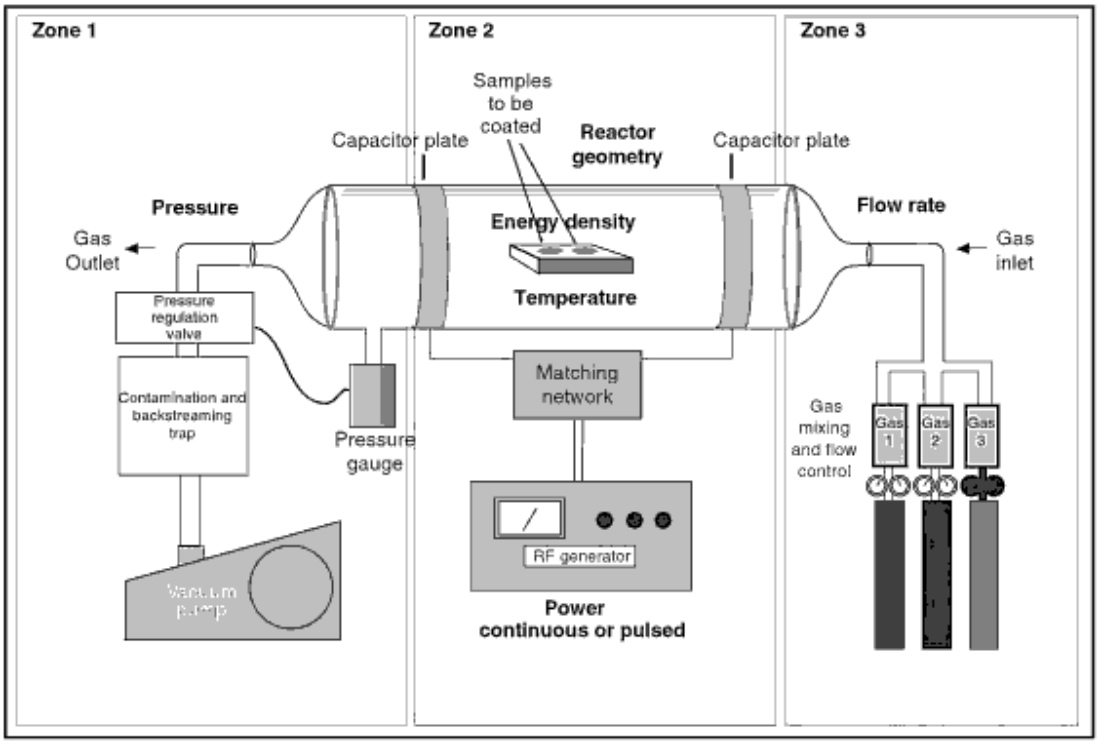

Figure 1.10 - A diagram of a capacitively coupled RF plasma reactor. Important experimental variables are indicated in bold typeface [52]

Several methods to modify the surface of materials are listed in table 1.4.

Table 1.4 - Physical and Chemical Surface methods [52]

	Polymer	Metal	Ceramics	Glass
Noncovalent coatings				
Solvent coating	✓	✓	✓	✓
Langmuir-active additives	✓	✓	✓	✓
Surface-active additives	✓	✓	✓	✓
Vapor deposition of carbons and metals	✓	✓	✓	✓
Vapor deposition of parylene (ρ-xylylene)	✓	✓	✓	✓
Covalently attached coating				
Radiation grafting(electron accelerator and gamma)	✓	-	-	-
Photografting (UV and visible sources)	✓	-	-	✓
Plasma (gas discharge)(RF, microwave, acoustic)	✓	✓	✓	✓
Gas-phase deposition				
Ion beam sputtering	✓	✓	✓	✓
Chemical vapor deposition	-	✓	✓	✓
Flame spray deposition	-	✓	✓	✓
Chemical grafting	✓	✓	✓	✓
Silanization	✓	✓	✓	✓
Biological modification(biomolecule immobilization)	✓	✓	✓	✓
Modifications of the original surface				
Ion beam etching (e.g., argon, xenon)	✓	✓	✓	✓
Ion beam implantation (e.g., nitrogen)	-	✓	✓	✓
Plasma etching (e.g., nitrogen, argon, oxygen, water vapor)	✓	✓	✓	✓
Corona discharge (air)	✓	✓	✓	✓
Ion exchange	✓	✓	✓	✓
UV radiation	✓	✓	✓	✓
Chemical reaction				
Nonspecific oxidation (e.g., ozone)	✓	✓	✓	✓
Functional group modifications (oxidation, reduction)	✓	-	-	-
Conversion coatings (photophating, anodization)	✓	✓	-	-
Mechanical roughening and polishing	✓	✓	✓	✓

1.7 Cytotoxicity tests

The absence of a toxic effect on cellular functions (cytotoxicity) is a prerequisite necessary for the biocompatibility of a material. For the determination of risk of a compound to human health, its evaluation at the cellular level, *in vitro*, is fundamental.

The *in vitro* studies are more adaptable, easily duplicated, inexpensive, simple, more reproducible and rapid. Currently, the testing of cytotoxicity effects of biomaterials *in vitro* is crucial for the development of new biomaterials. [53], [54]

The selection of the cell line type to perform materials cytotoxic profile is essential. The choice of cell type (osteoblasts, fibroblasts, endothelial cells) depends on the future application intended for the tested materials. [53]

The MTS assay, namely 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, is a colorimetric method that allows the determination of the percentage of viable cells seeded in the presence of a test material. In viable cells, metabolism generates “reducing equivalents” like NADH and NADPH. These “reducing equivalents” transfer their electrons to an intermediate electron transfer reagent that could reduce the tetrazolium product (MTS), into an aqueous, soluble formazan product. Creation of the colored formazan product is proportional to the quantity of viable cells in culture. At death, therefore, cells quickly lose the ability to reduce tetrazolium products. [126], [56]

1.8 Characterization techniques

1.8.1 Contact Angle

Systems which contain liquids and solids are everywhere. Bringing a liquid to contact a solid surface is a method that is called as wetting. This is process has fascinated scientific attention over more than 2 centuries. [62]

The hydrophilicity or hydrophobicity of a solid surface can be determined by measurements of the contact angle created between water, air and that surface. Figure 1.11 shows that when the surface is hydrophobic the water droplet produces

compact globs with large contact angles. When the surface is hydrophilic water droplet spread to produce flattened globs with small contact angles. [63]

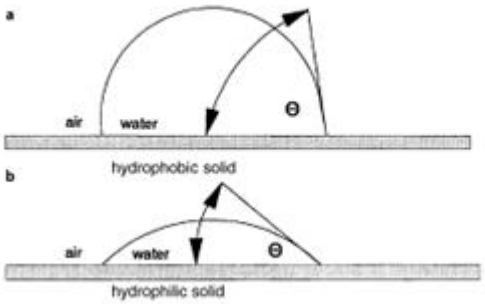

Figure 1.1 - Contact angles formed between water droplets and a) hydrophobic surface and b) hydrophilic surface [63]

This method use a goniometer-microscope armed with an angle-measuring eyepiece, or more newly, a video camera armed with a suitable magnifying lens, connected with a computer with image analysis software to find out the tangent value exactly on the captured image. [64]

The contact angle is measured according to the young equation given below (figure 1.12):

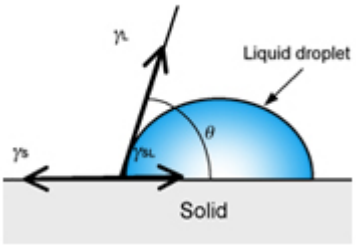

Figure 1.2 - Schematic representation of a water droplet in a surface and the forces present [127]

An angleθ, which is called contact angle, is formed when a drop of a liquid is positioned on the +solid surface. Young has revealed that: [65]

Equation 2.1 $\gamma_S = \gamma_{SL} + \gamma_{LV}.\cos\theta$,

Where γ_S, γ_{SL} and γ_{LV} are respectively the surface free energy of the interface between the solid and saturated vapor, between the solid and the liquid and the liquid and the vapor. Different methods have been suggested to obtain γ_S using the contact angles formed by drops of various liquid with known surface tensions.

The contact angle does not measure directly the surface free energy. The wettability of solids is mostly influenced by the surface free energy. It means that the surface free energy can only be estimated in an indirect way, if the wettability is quantitatively measured. [66]

The energy of adhesion could be separated into different contributions. Most valuable for a lot of applications is the division between disperse and polar interactions. [67]

Equation 2.2 $W = W^{disperse} + W^{polar}$,

Where, $W^{disperse}$ is exclusively based on London forces which means on interactions occurring between instantaneous dipoles and ^{Wpolar} is relative to molecules with static dipole moment. [67]

The surface free energy is expressed by: [67]

Equation 2.3 $\gamma_S = \gamma_S^d + \gamma_S^p$,

Where,

γ_S is the surface energy of the solid

γ_S^d is the dispersive component of surface energy

γ_S^p is the polar component of surface energy

On table 1.5, is shown the liquids usually used for contact angle measurements and their surface tensions. [65]

Table 1.5 - Surface free energies of liquids used for contact angle measurements (liquids are ordered as a function of its polarity degree x^p) [65]

Liquid	γ_{LV} (mJ/m^2)	γ_{LV}^d (mJ/m^2)	γ_{LV}^p (mj/m^2)	x^p(%)
Water	**72.2**	**22.0**	**50.2**	**69.5**
Glycerol	**64.0**	**34.0**	**30.0**	**46.9**
Formamide	**58.3**	**32.3**	**26.0**	**44.6**
Ethan-1.2-diol	**48.3**	**29.3**	**19.0**	**39.3**
Polyglycol E-200	**43.5**	**28.2**	**15.3**	**35.2**
Polyglycol 15-200	**36.6**	**26.0**	**10.6**	**29.0**
Dimethylsulphoxide	**43.6**	**34.9**	**8.7**	**20.0**
2-Ethoxyethanol	**28.6**	**23.6**	**5.0**	**17.5**
Dimethylformamide	**37.3**	**32.4**	**4.9**	**13.1**
Trieresylphosphate	**40.7**	**36.2**	**4.5**	**11.1**
Di-iodomethane	**50.8**	**48.5**	**2.3**	**4.5**
Pyridine	**38.0**	**37.2**	**0.0**	**2.1**
Hexadecane	**27.6**	**27.6**	**0.0**	**0**
Tetradecane	**26.7**	**26.7**	**0.0**	**0**
Dedecane	**25.4**	**25.4**	**0.0**	**0**
Decane	**23.9**	**23.9**	**0.0**	**0**
Octane	**21.8**	**21.8**	**0.0**	**0**
Hexane	**18.4**	**18.4**	**0.0**	**0**

In the next section, the most common methods used for the determination of the surface free energy (SFE) will be discussed.

Zisman method

Zisman studied empirically that a plot of cos θ versus $\sigma_{L,V}$ is always linear. The extrapolation of which cos θ to 1 is denominated as the critical surface tension. The equation came from this empirical experience:

Equation 2.4 $\cos\theta = 1-K(\sigma_{L,V}-\sigma_c)$,

Where $\sigma_{L,V}$ is the surface tension of the liquid and σ_c is the critical surface tension of the solid. However, this method is only valid to pure liquids. [68]

Zisman and coworkers settled an empirical connection between the cosine of the contact angle cos θ and the liquid/air interfacial tensionγ. They calculated the contact angle θ for many different liquids, for a given low energy solid surface. [69]

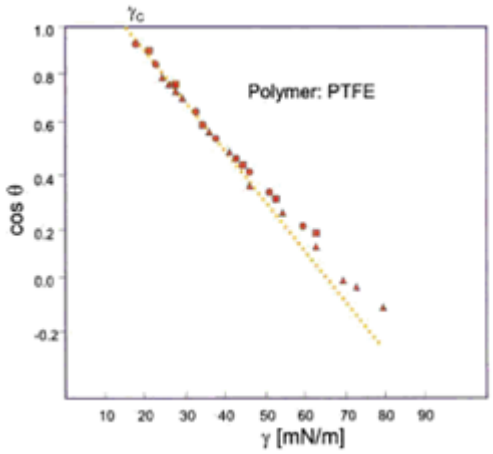

Figure 1.13 - Zisman plot of the contact angle of different liquids on a PTFE surface [69]

Fowkes

In 1964, Fowkes has demonstrated that the work of adhesion, W_c and the work of adhesion, W_a, can be divided into their dispersion, d, polar, p, induction, i, and hydrogen-bonding, h components: [64]

$W_c = W_c^d + W_c^p + W_c^i + W_c^h + \ldots$

$W_a = W_a^d + W_a^p + W_a^i + W_a^h + \ldots$

The work of the dispersion component between a solid and a liquid could be formulated as: [64]

Equation 2.5

$$W_a^d = \sqrt{\left(W_c^d\right)_S \left(W_c^d\right)_{LV}} = 2\sqrt{\gamma_S^d \, \gamma_{LV}^d}$$

The interaction between purely dispersive molecules was calculated by Fowkes, to be a geometric mean. The interfacial interactions could be illustrated (by using a purely dispersive liquid→ γ_{LV}^{nd}=0) as: [70]

Equation 2.6 $$\gamma_{SL} = \gamma_{SV} + \gamma_{LV} - 2\sqrt{\gamma_{SV}^{d}\ \gamma_{LV}^{d}}$$

Combining the Eq. 2.6 with Young´s equation is obtained the eq. 2.7: [70]

Equation 2.7 $$\cos\theta = 2\sqrt{\gamma_{SV}^{d}} \cdot \frac{1}{\sqrt{\gamma_{LV}^{d}}} - 1$$

The Owens, Wendt, Rabel and Kaelble method

This method considers that the interfacial energy could be divided between molecules according to the interaction forces: polar interactions between permanent dipoles or permanent dipole-induced dipole and dispersive interactions between nonpolar molecules in which temporary fluctuations occur. [71]

The interfacial energy could be calculated through a geometric mean of the contributions of the liquid and solid, according to the equation 2.8: [71]

Equation 2.8 $$\gamma_{SL} = \gamma_{S} + \gamma_{l} - 2\sqrt{\gamma_{S}^{d} \cdot \gamma_{l}^{d}} + \sqrt{\gamma_{s}^{p} \cdot \gamma_{l}^{p}}$$

Combining the Eq.2.8 with Young´s equation is obtained the Eq.2.9: [71]

Equation 2.9 $$\gamma_{LV}(1 + \cos\theta) = 2\sqrt{\gamma_{S}^{d} \cdot \gamma_{l}^{d}} + \sqrt{\gamma_{s}^{p} \cdot \gamma_{l}^{p}}$$

Wu´s method (harmonic-mean approach)

This method utilizes a harmonic-mean equation for the sum of the dispersion and polar contributions. Wu declared that the Owens and Wendt equation was giving surface tensions for polymers with an error of 50-100% when compared with their melt values, mostly for polar polymers. However, the harmonic-mean approach has

the same faults as the Owens and Wendt approach, because the cohesive polar interactions properties cannot resolve the interfacial interaction among two distinctive materials. [64]

In table 1.6, is shown the main characteristic of the method discussed previously.

Table 1.6 - Calculation of Surface Free Energy [72]

Method	Information	Min.no.of liquids	Application	Examples
Zisman	**Critical Surface Tension**	**2**	**Non-polar solids**	**PE, PTFE, Waxes**
Fowkes	**Disperse parts of surface free energy**	**2, non-polar liquids**	**Non-polar system**	**PE, PTFE, Waxes**
Wu	**Disperse and polar parts of surface free energy**	**2, at least one polar liquid**	**Low energetic systems**	**Organic solutions, polymers, organic pigments**
WORK	**Disperse and polar parts of surface free energy**	**2**	**Universal**	**Polymers, aluminum, coating, vanishes**

1.8.2 SEM (scanning electronic microscopy)

SEM (scanning electron microscope) was designed at the RCA Laboratories in New Jersey, under wartime conditions, fundamented on secondary emission of electrons. [76]

A major advantage of the SEM for surface observations is that sample preparation is generally simple. In the simplest case, the material to be examined, chosen carefully from a larger sample, is placed on double sided sticky tape on a specimen stub. [77]

The observation and characterization of heterogeneous organic and inorganic materials on a nanometer (nm) to micro micrometer (μm) scale is possible with the

scanning electron microscope (SEM). SEM can provide three-dimensional-like images of the surface of a very large range of materials. The main use of the SEM is to achieve topographic images in the magnification range 10-10,000x. [78]

The main components of an SEM are presented in the figure 1.14.

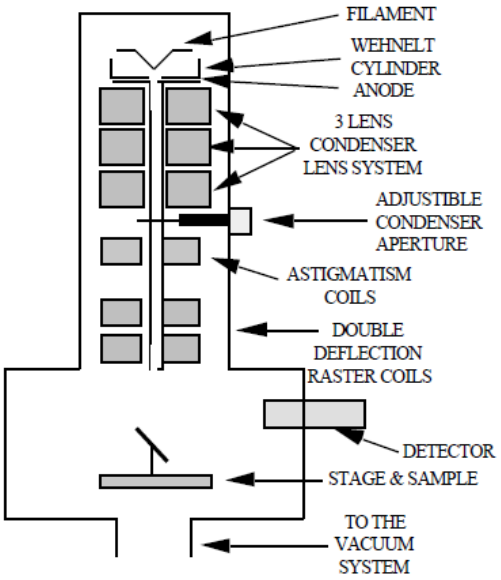

Figure 1.14 - Diagram of SEM column and specimen chamber [79]

Vacuum, beam generation beam manipulation, signal processing and display, beam interaction, detection, and record are the seven primary operation systems. The results and qualities of a micrograph such as magnification, resolution, brightness, contrast and depth of field are calculated by these operation systems. [79]

One of the tools commonly used in semiconductor materials and device research is SEM. The sample, to avoid burning and damaging, has to be coated (conductive material). When a fine beam of electrons is scanned through the surface of a specimen, a detector monitors the intensity of secondary electron emission from the specimen. On a screen a spot is shown, which (the point) is scanned in synchronism with the scanning electron beam on the specimen. The detected signal amplitude is responsible for the brightness of the spot. When the intensity of the

emitted secondary signal changes through the specimen, the same contrast pattern is displayed in the SEM image. [80]

1.8.3 Swelling/Degradation

1.8.3.1 Swelling

In the absence of reactions, when implant materials touch a biological system, occurs tissue interface. If the primarily fluid - the substance, goes from the tissue into the biomaterial, there is an increase of the volume of the material due to the conservation of the volume. This phenomenon is called swelling, which could cause a large deformation in materials and affect material´s mechanical properties. Swelling creates continual deformation, which can lead to a mode of failure. [84]

The swelling ratio of a sample establishes its capacity to swell following absorption of water and is a significant parameter for sample use. The next equation was estimated to know the swelling ratio: [85]

Equation 2.10 $$\% \text{ swelling} = \left(\frac{Ws - Wd}{Wd}\right) * 100,$$

Where, Wd and Ws are respectively the weights of the samples in the dry and swollen states.

The ability of swelling also depends on environmental conditions such as salt concentration, pH and temperature of the medium where they are. [86]

1.8.3.2 Degradation

The changes in the chemical structure and physical properties of the polymers caused by external chemical or physical stresses due to chemical reaction are denominated as degradation. Polymer degradation consists of oxidation, pyrolysis, biodegradation, photo-catalytic and mechanical degradation. Taking into account their chemical structure, polymers are susceptible to dangerous effects from the environment, which includes chemical deteriogens like humidity, dangerous anthropogenic emission, oxygen (its actives forms) and atmospheric pollutants and

physical stresses like mechanical forces, ablation, radiation and heat. [87]. This latter could affect the main chain linkages, the substituent atoms and side chain of the polymers. [88]

1.8.4 Fourier Transform Infrared Spectroscopy (FTIR)

To identify chemicals (organic and inorganic), FTIR is most helpful. It might be used to quantitate some elements of an unknown mixture, and to analysis gases, liquids and solids. The method in which data is gathered and converted from an interference pattern to a spectrum is designate by Fourier Transform Infrared Spectroscopy (FTIR) and it is a very recent development. [73], [74] In table 1.7, is showed the advantages and disadvantages of this technique.

Table 1.7 - The advantages and disadvantages of FTIR [81]

Advantages	Disadvantages
Almost Universal	Can´t detect some molecules
Spectra are information rich	Mixtures
Relatively fast and easy	Water
Relatively inexpensive	
Sensitivity	

Molecular rotations and vibrations of chemical elements absorb specific frequencies of electromagnetic waves. The infrared, a particular part of the electromagnetic spectrum, is mainly appropriate for the detection of molecular vibrations. The infra-red spectrum relates to electromagnetic waves whose wavelengths range from 0.78 μm to 1000 μm. [75]

The molecular vibrations might be divided into two categories: stretching and bending. Stretching vibrations are classified asymmetric or asymmetric and bending vibrations categorized as rocking, scissoring, wagging or twisting (figure 1.15) [75]

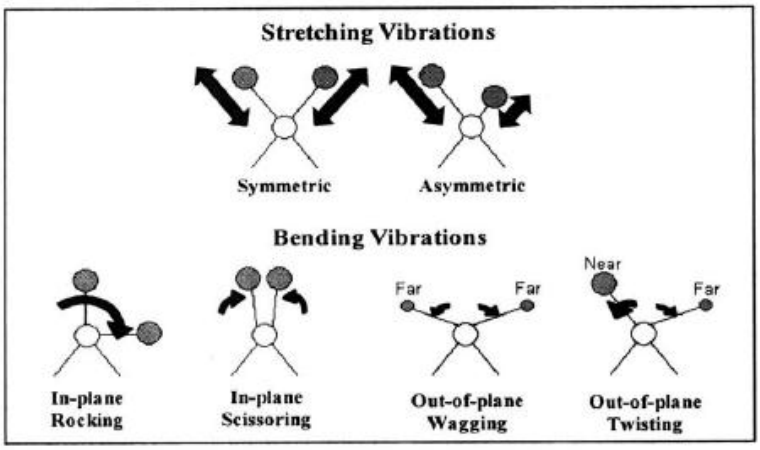

Figure 1.15 - Types of molecular vibrations [75]

1.9 Hydrophobic recovery

After surface modification, PDMS films regain its hydrophobic nature, in a process called "hydrophobic recovery". [89]

The possible mechanisms responsible for hydrophobic recovery of silicone rubbers were summarized by Owen *et al* and are listed below: [90], [91]

- ➔ External contamination of the surface;
- ➔ Changes in surface roughness;
- ➔ Condensation of silanol groups at the surface;
- ➔ Reorientation of polar groups from the surface into the bulk;
- ➔ Diffusion of low molecular weight species (LMWS) from the bulk to the surface;

The latter point is the main process responsible for hydrophobic recovery. LMWS are identified as a homologous series of cyclic oligomeric dimethylsiloxanes of the general formula Dn= [(CH32SiO)], where n is the number of repeating units. The cause of low molecular weight species in the elastomer are incomplete curing, partial discharge induced reactions and the addition of silicone liquids as processing aids and ultra –violet. [90]. The formation of a hydrophilic silica-like surface layer after surface modification retards the migration of low molecular weight species to the surface. But, the hydrophobic recovery could increase by the diffusion of low molecular weight species into the surface caused by the cracking of the SiO_x layer, as illustrated in figure 1.16.

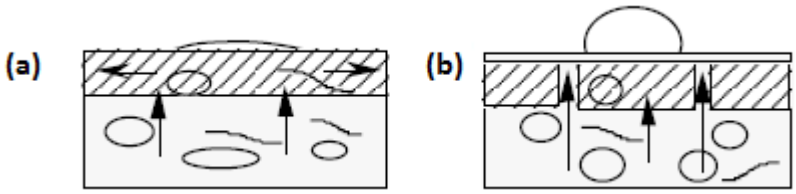

Figure 1.16 - Transport of low molar molecular mass siloxanes through a continuous (a) or cracked (B) silica-like surface layer [91]

2. Materials and methods

2.1 Reagents

The reagents used during the experimental activity, are shown below:

- Acetone
- Methacrylic acid
- Hydroxyethylmethacrylate
- Irgacure® 2959
- Sylgard 184®
- Formamide
- Diiodomethane
- Distilled water
- Milli q water
- Potassium hydroxide
- Ethanol
- 1,6-hexanediamine
- Propanol
- Phosphate-buffered saline solution (PBS)
- Fetal bovine serum (FBS)
- Dulbecco's modified Eagle's medium (DMEM-F12)
- Ethylenediaminetetraacetic acid (EDTA)
- L-glutamine
- Penicillin G
- Human Fibroblast Cells
- Streptomycin
- Amphotericin B
- Trypsin
- *Escherichia coli* (*E. coli*) DH5α
- Lysogeny broth (LB) agar

2.2 Preparations of PDMS films

Sylgard®184 (PDMS) kit was supplied by DOW-Corning, consisting of a base and a curing agent. These 2 components were thoroughly mixed, using a rate of 10:1, by mass, and after that, degassed under vacuum. Films (with 0.5mm of thickness) were vulcanized 4 hours, at 65ºC, and then washed narrowly with acetone. (Figure 2.1)

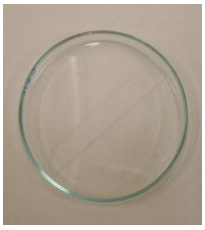

Figure 2.1 - Film of PDMS.

2.3 Cytotoxicity tests

In this study, human fibroblasts as model cells in the evaluation of materials cytotoxicity. Fibroblasts produce the extracellular matrix to which cells adhere. This structural framework is fundamental for animal tissue formation. These types of cells are the most common cells of connective tissue and have a crucial role in wound healing. [55] These cells were chosen to perform the cytotoxicity studies, because they are easy to maintain in culture and the results obtained *in vitro* show a good correlation with those obtained *in vivo.*

Dulbecco's modified Eagle's medium (DMEM-F12), ethylenediaminetetraacetic acid (EDTA), L-glutamine, 3-(4,5-dimethylthiazol-2-yl)-5-(3carboxymethoxyphenyl)-2-(4-sulphofenyl)-2H-tetrazolium, inner salt (MTS), penicillin G, phosphate-buffered saline solution (PBS), streptomycin, amphotericin B and trypsin were purchased from Sigma-Aldrich (Sintra, Portugal). Human Fibroblast Cells (Normal Human Dermal Fibroblasts adult, criopreserved cells) were purchased from PromoCell (Labclinics, S.A.; Barcelona, Spain). Fetal bovine serum (FBS) was purchased from Biochrom AG (Berlin, Germany).

Human Fibroblasts cells were seeded in T-flasks of 25 cm^2 with 6 mL of DMEM-F12 supplemented with heat-inactivated FBS (10% v/v) and 1% antibiotic/antimycotic solution. After the cells become confluent, they were subcultivated by a 3-5 minutes incubation in 0.18% trypsin (1:250) and 5mM EDTA. Subsequently, cells were centrifuged, resuspended in culture medium and then seeded in T-flasks of 75 cm^2. Hereafter, cells were kept in culture at 37 °C in a 5% CO_2 humidified atmosphere, inside an incubator. To evaluate cell behaviour in the presence of the materials,

fibroblasts cells were seeded with materials in 96-well plates at a density of $10x10^3$ cells per well, for 96 hours. The materials were sterilized by UV irradiation for 30 minutes, before being placed in contact with cells. Cell growth was monitored using an Olympus CX41 inverted light microscope (Tokyo, Japan) equipped with an Olympus SP-500 UZ digital camera. [57], [58]

Human fibroblasts cells were seeded in the presence of materials, in 96-well plate, with 100 µl of DMEM-F12 and following incubated at 37°C, in a 5% CO_2 humidified atmosphere. After an incubation period (24, 48, 72 and 96 hours), cell viability was assessed through the reduction of the MTS into a water-soluble formazan product. Briefly, the medium of each well was removed and replaced with a mixture of 100µL of fresh culture medium and 20µL of MTS/PMS reagent solution. Then, cells were incubated for 4 hours at 37°C, under a 5% CO_2 humidified atmosphere. The absorbance was measured at 492 nm using a microplate reader (Sanofi, Diagnostics Pauster). Wells containing cells in the culture medium without materials were used as negative controls (K^-). EtOH (96%) was added to wells that contained cells, as a positive control (K^+). [59], [60], [61]

The obtained results were expressed as the mean ± the standard error of the mean (n=4). Statistical significance was calculated using a one-way analysis of variance (one-way ANOVA) and differences between groups were tested by a one-way ANOVA with Dunnets post hoc test.

2.4 Determination of PDMS materials antibacterial activity

Bacterial strain *Escherichia coli* (*E. coli*) DH5α was purchased from ATCC and LB agar was purchased from Pronadise.

2.5 Characterization techniques

2.5.1 Contact Angle

In this work, the surface free energy was determined by measuring the water contact angle with different solvents: water distilled, Formamide and Diiodomethane.

The determination of the surface free energy and respective polar and disperse components was made using the method OWRK.

2.5.2 SEM (scanning electronic microscopy)

SEM was used to determine the PDMS materials antibacterial activity. Firstly the bacteria *E.coli* was allowed to grow in an agar plate in the presence of the materials for 24h. After the biofilms were examined using standard methods to treat the biofilm prior to imaging (SEM analysis). Briefly, the biofilm samples were immersed in 2.5% glutaraldehyde overnight in order to preserve the structure of living tissue with no alternation from the living state. After the primary fixation with glutaraldehyde, the biofilm samples were dehydrated with increasing concentrations of ethanol, 50%, 70%, 90% and 100%. Subsequently, the materials were mounted on stubs using a double-side adhesive -tape and sputter coated with gold using an Emitech K550 sputter coater (London, UK). The SEM images were acquired with a scanning electron microscope Hitachi S-2700 (Tokyo, Japan) with an acceleration voltage of 20 kV at different magnifications. [83]

2.5.3 Swelling/Degradation

In this work, were placed small pieces of PDMS corresponding to the various techniques in the oven, under vacuum, at 37 ° C for one day until constant weight. After one day, these small pieces were weighed and subsequently placed in different storage mediums – distilled water, phosphate-buffered saline solution (PBS) and growth medium. The percentage of swelling was calculated over 5 hours, 1 day, 1 week and 1 month.

2.6 Hydrophobic recovery

To analyze the hydrophobic recovery time over time, were placed small pieces of PDMS corresponding to the various techniques in the oven, under vacuum, at 37 ° C for one day until constant weight. After one day, the water contact angle was

measured and subsequently these small pieces were placed in different storage mediums – distilled water, phosphate-buffered saline solution (PBS) and growth medium. Then, the water contact angle was calculated along time: 5 hours, 1 day, 1 week and 1 month.

3. Surface Modification by UV

For more than 50 years, radiation graft polymerization has been commonly used for polymer chemistry. Normally, this technique involves the production of radicals (reactive sites) on the polymer surface followed by the covalent linkage of a preconceived polymer, or, more typically, by the polymerization of a monomer from those reactive sites. [92]

The addition of an acid in the monomer solution, in UV systems, increases grafting yield and enhances homopolymer formation in a similar way to the ionizing work. [93]

A photo-initiator can induce graft polymerization in the proper UV radiation range. [94] Irradiation with UV lamps and/or pulsed laser might also increase the surface proprieties of polymers, providing advantages like large radiation area, high power density, low fabrication temperature and short reaction time. [95]

Irgacure 2959® (4-(2-hydroxyethoxy)phenyl-(2-propyl) ketone, as illustrated in figure 3.1, is the most popular photoinitiator for UV curing systems and can be used in photopolymerization of polymers and copolymers. This photoinitiator is very used for tissue engineering applications due to its solubility and its minimal toxicity when compared to other Irgacure type photoinitiator. [96], [97]

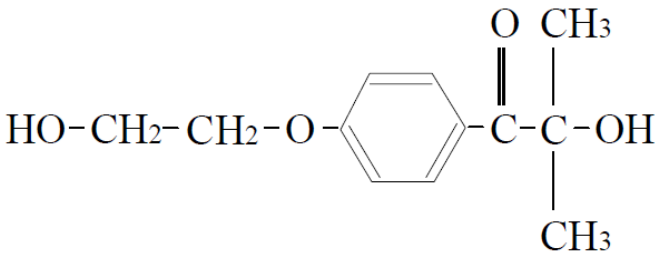

Figure 3.1 - Structural formula of Irgacure 2959® [98]

Upon absorption of UV light, Irgacure 2959® separates into free radicals [99], according to the figure 3.2:

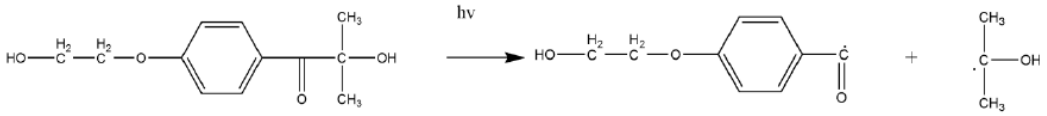

Figure 3.2 - Free radical formation from I-2959® due to UV light exposure [100]

These free radicals will enable the hydrogen abstraction from PDMS methyl group, generating a radical on PDMS surface capable of initiating the polymerization of acrylic monomers, as illustrated in figure 3.3. [99]

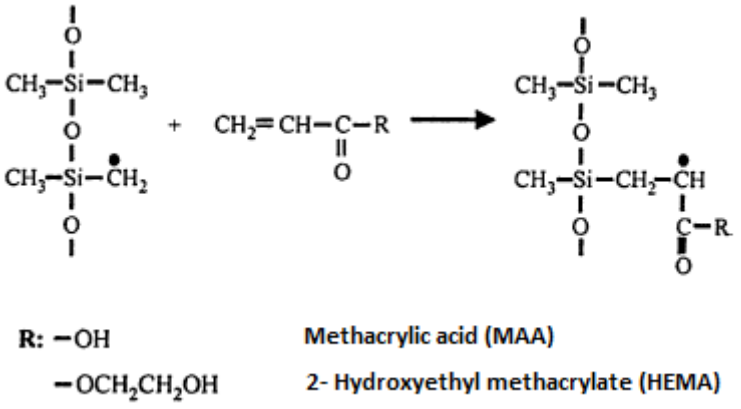

Figure 3.3 - Initiation of the polymerization reaction, where R is the monomer side group. [99]

3.1 Modification technique

PDMS films were previously activated by UV light (using an UV lamp UVGL 48), in the 254nm wavelength setting, in an aqueous solution of Irgacure® 2959 (photoinitiator given by CIBA), for 30 minutes. After removing the silicone films from the solution of Irgacure, two procedures were adopted. In the first procedure, the silicone films were added to a 10% (v/v) MAA (Methacrylic acid) aqueous solution, or to a 10% (v/v) HEMA (Hydroxyethylmethacrylate) aqueous solution during 15, 30, 60, 120, 180 and 240 minutes. In the second procedure, the silicone films were added to a 10% (v/v) MAA (Methacrylic acid) aqueous solution, or to a 10% (v/v) HEMA (Hydroxyethylmethacrylate) aqueous solution and then were irradiated with UV light, during 15, 30, 60, 120, 180 and 240 minutes.

3.2 Results

Water contact angles were measured for unmodified PDMS films and modified films, either with HEMA or MAA, without using UV radiation after the previously activation with UV light in an aqueous solution of Irgacure® 2959, as illustrated in figure 3.4. (First procedure)

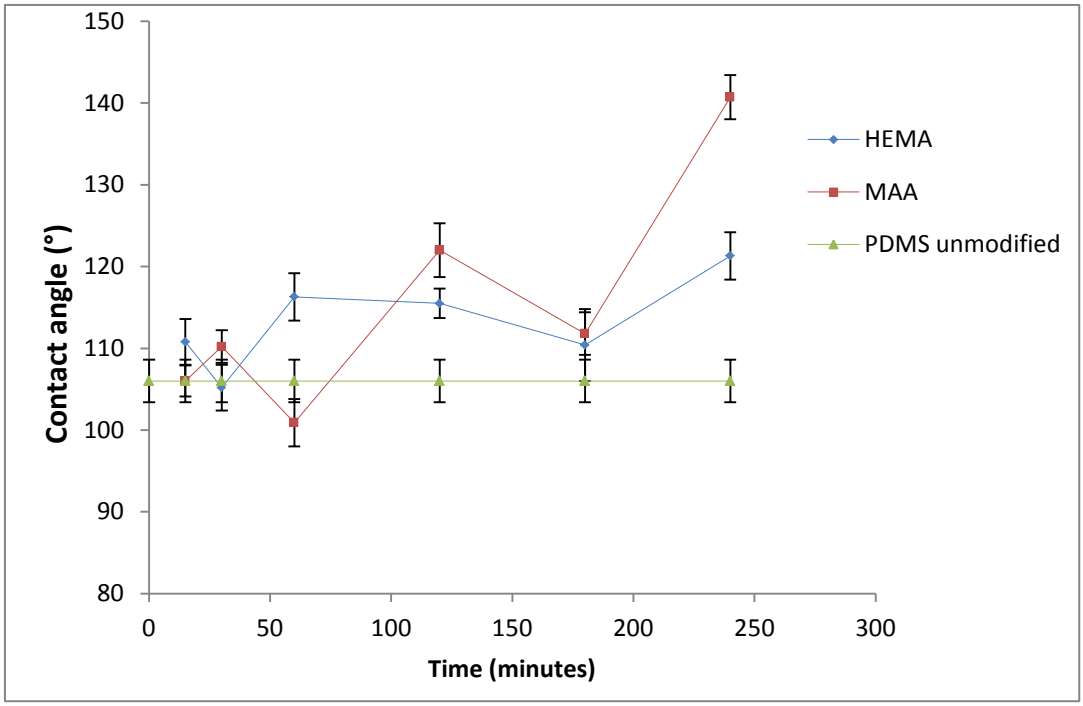

Figure 3.4 – Variation of water contact angles, along time, without using UV radiation after the previously activation with UV light in an aqueous solution of Irgacure® 2959 (First procedure)

It was also measured the water contact angle when PDMS was modified by UV, either with HEMA or MAA for different irradiation times, as illustrated in figure 3.5. (Second procedure)

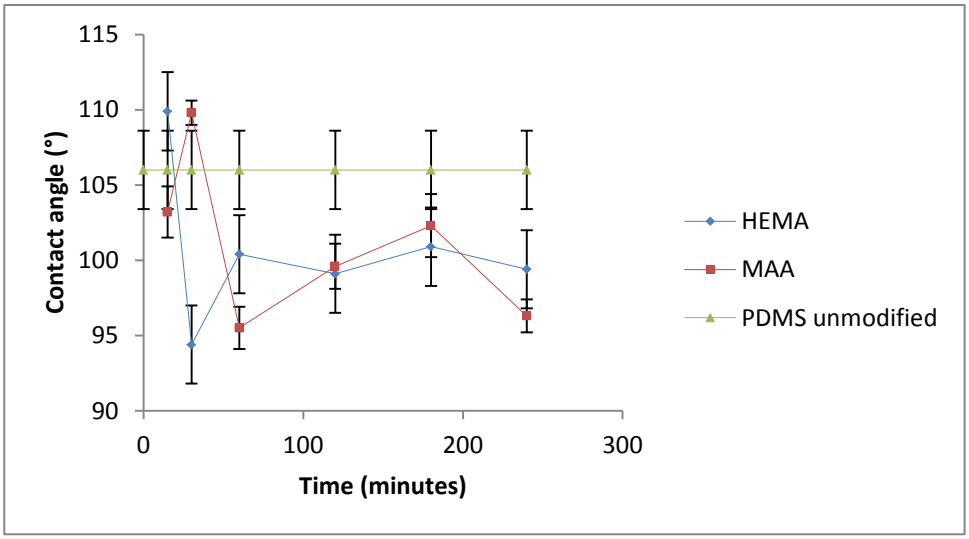

Figure 3.5 - Variation of water contact angles, along time, after modification with HEMA and MAA in UV. (Second procedure)

The lowest water contact angle value was obtained after 30 min and 1 hour for HEMA and MAA, respectively (the smaller the water contact angle is, more hydrophilic the PDMS surface becomes). Figure 3.5 shows better results than figure 3.4, regarding the decrease of the water contact angle, which leads to the conclusion that the modification of PDMS films using UV radiation after the previously activation with UV light in an aqueous solution of Irgacure® 2959, provides a more hydrophilic character to the PDMS surface. Considering these times as ideal for grafting (30min for HEMA and 1h for MAA), the remaining characterization techniques (surface free energy, swelling / degradation and of hydrophobicity recovery) will only be made for these selected conditions. Finally, for the same selected conditions, the antibacterial activity and cytotoxicity of the PDMS films were evaluated.

3.2.1 Surface free energy

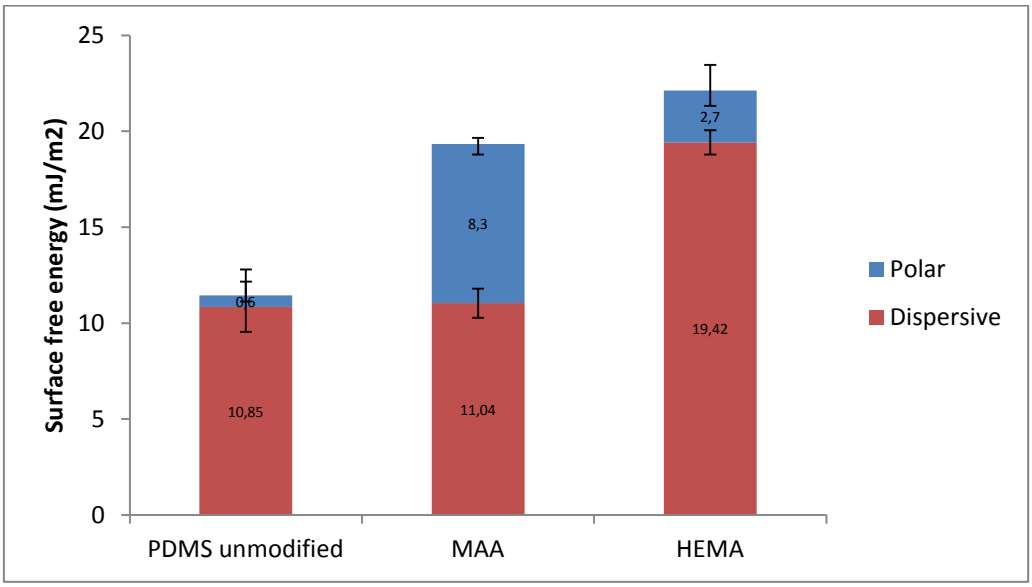

Figure 3.6 - Surface free energy of PDMS unmodified and pdms modified with HEMA and MAA

Figure 3.6 shows that the incorporation of the HEMA or MAA in the surface of the films brought an increase of the surface free energy. The increase of the polar component can be explained to the grafted polar groups (C=O in ester groups, in hydroxyl groups as C-OH, and ether carbon bonds as C-O) on PDMS surface. The polar component of the PDMS surface grafted by HEMA is lower than expected, because sometimes HEMA behave as a hydrophobic polymer (the OH groups are wrapped in methyl groups).

3.2.2 Swelling/Degradation

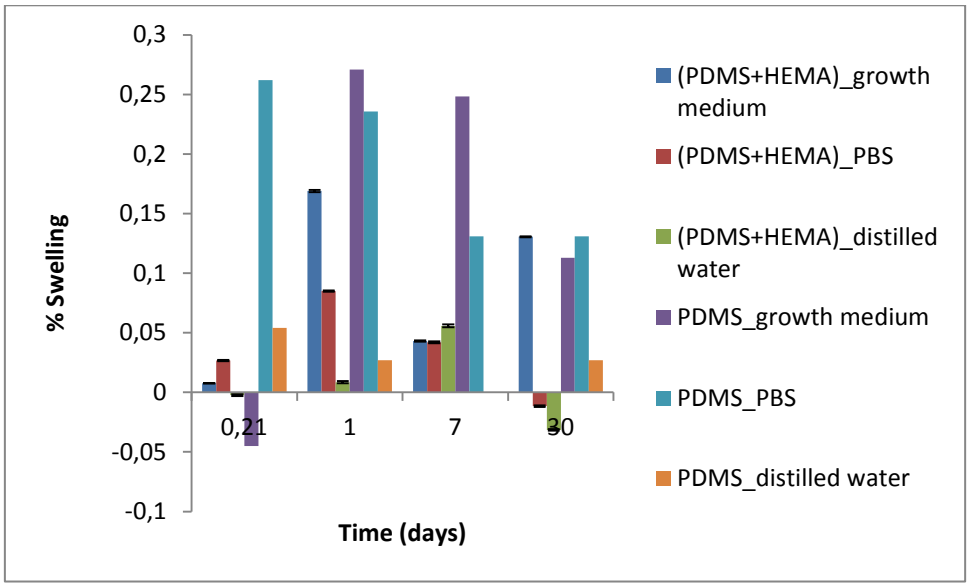

Figure 3.7 - Percentage of swelling after the modification of PDMS by HEMA, under different storage mediums, along time

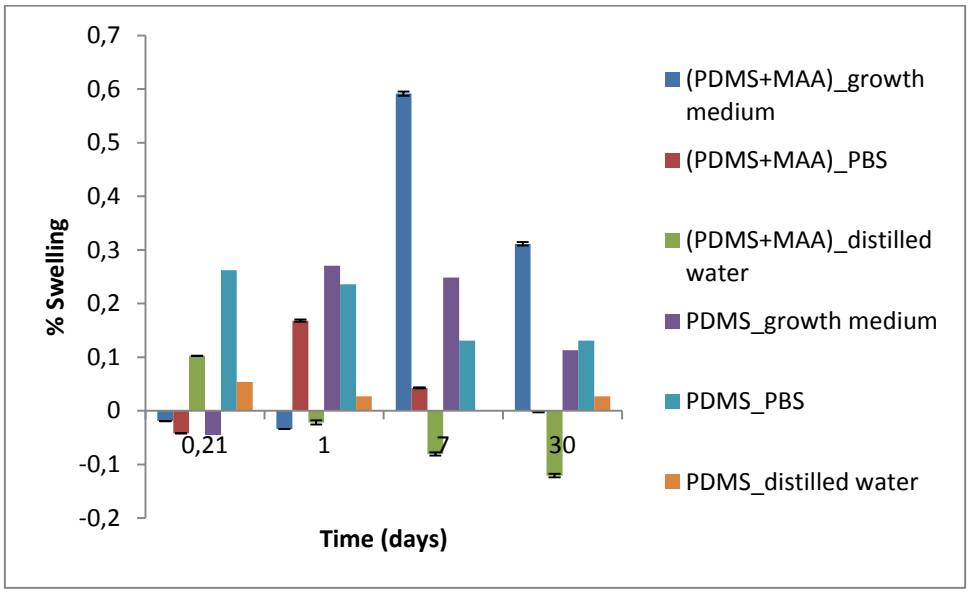

Figure 3.8 - Percentage of swelling after the modification of PDMS by MAA, under different storage mediums, along time

As expected, figure 3.7 and 3.8 show that, there wasn't degradation, neither swelling on PDMS unmodified and on PDMS modified with HEMA and MAA. After surface modification by UV, silicon films remained highly reticulated and therefore the percentage of swelling/degradation is very low.

3.2.3 Hydrophobicity recovery

In this analysis (hydrophobicity recovery) is presented the effects of storage conditions on maintaining hydrophilic behavior. This method was evaluated by determination of the water contact angle of surface modified PDMS by UV, using HEMA and MAA (figures 3.9 and 3.10, respectively)

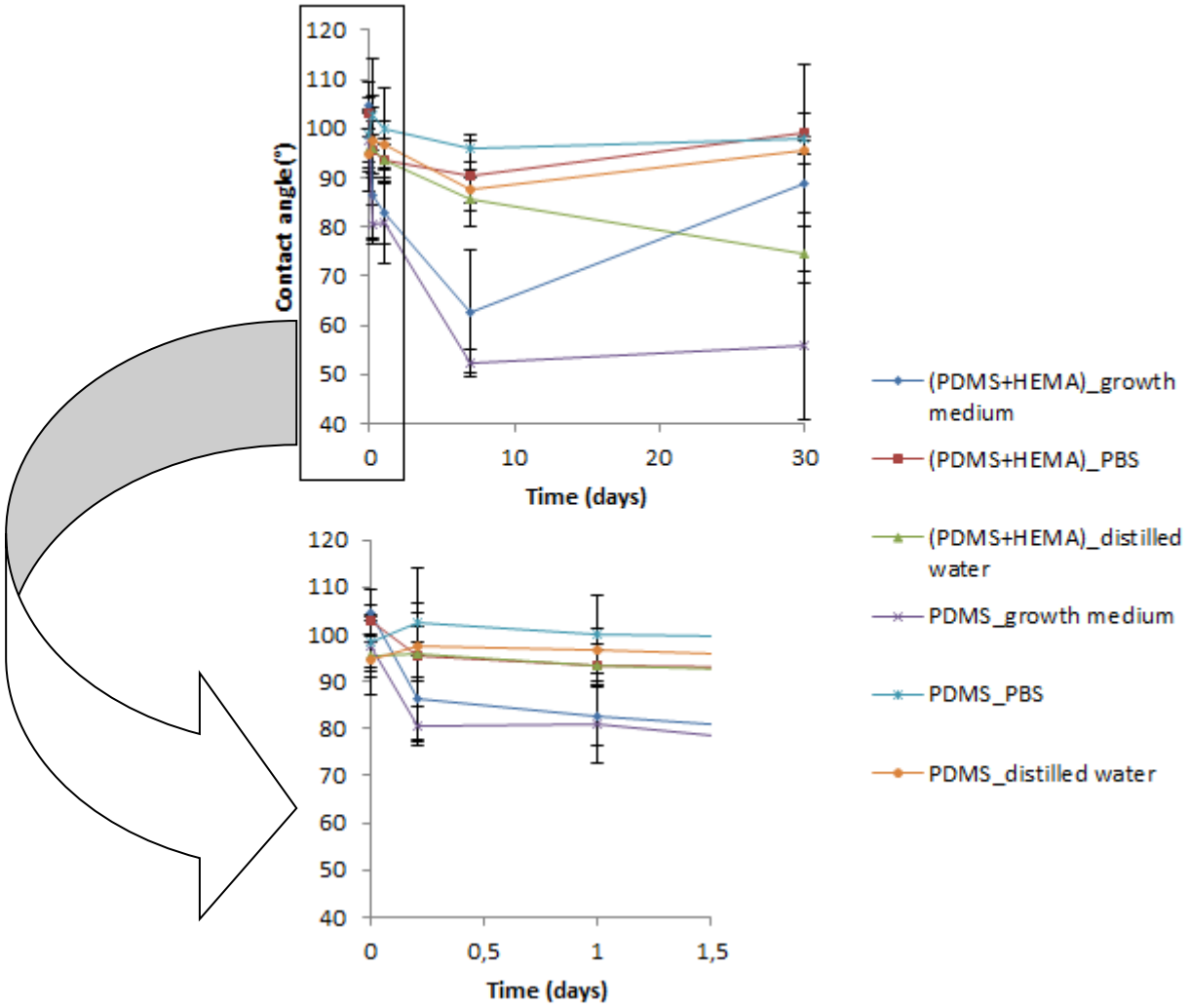

Figure 3.9 - Contact angle after UV modification of PDMS with HEMA, under different storage mediums, along time

After the surface modification, the behavior of the storaged samples showed to be different. During the first week there was an accentuated decrease of the contact angle and then the hydrophobicity recovery was observed after the 5 hours, using the growth medium as a storage medium. Growth medium contains salt and proteins. The

protein molecules have sufficiently high molecular weight so do not affect the hydrophobic recovery. Growth medium contains a number of nutrients that are used to provide an appropriate biochemical environment in cell and tissue culture applications. The decrease of the contact angle on PDMS surface when storaged in growth medium could be explained by the deposition of amino and carboxyl groups on the surface, from the amino acids present in the growth medium. Using PBS as storage medium, the behavior was similar to the one observed in the growth medium but the increase of the water contact angle was less accentuated. Distilled water proved to be the most effective way to reduce / maintain the hydrophilicity of the films (after modification) since after one month the contact angle showed to be the lowest. The use of distilled water could lead to the hydrolysis of siloxane bonds (creating hydrophilic silanol groups), or surface erosion and reduction of fillers in the surface region. Water penetrations into the surface and/or reorientation of polar groups are also, the causes of the decreased hydrophobicity. [91] However, after one month, the lowest water contact angle is verified on PDMS unmodified, when stored in growth medium.

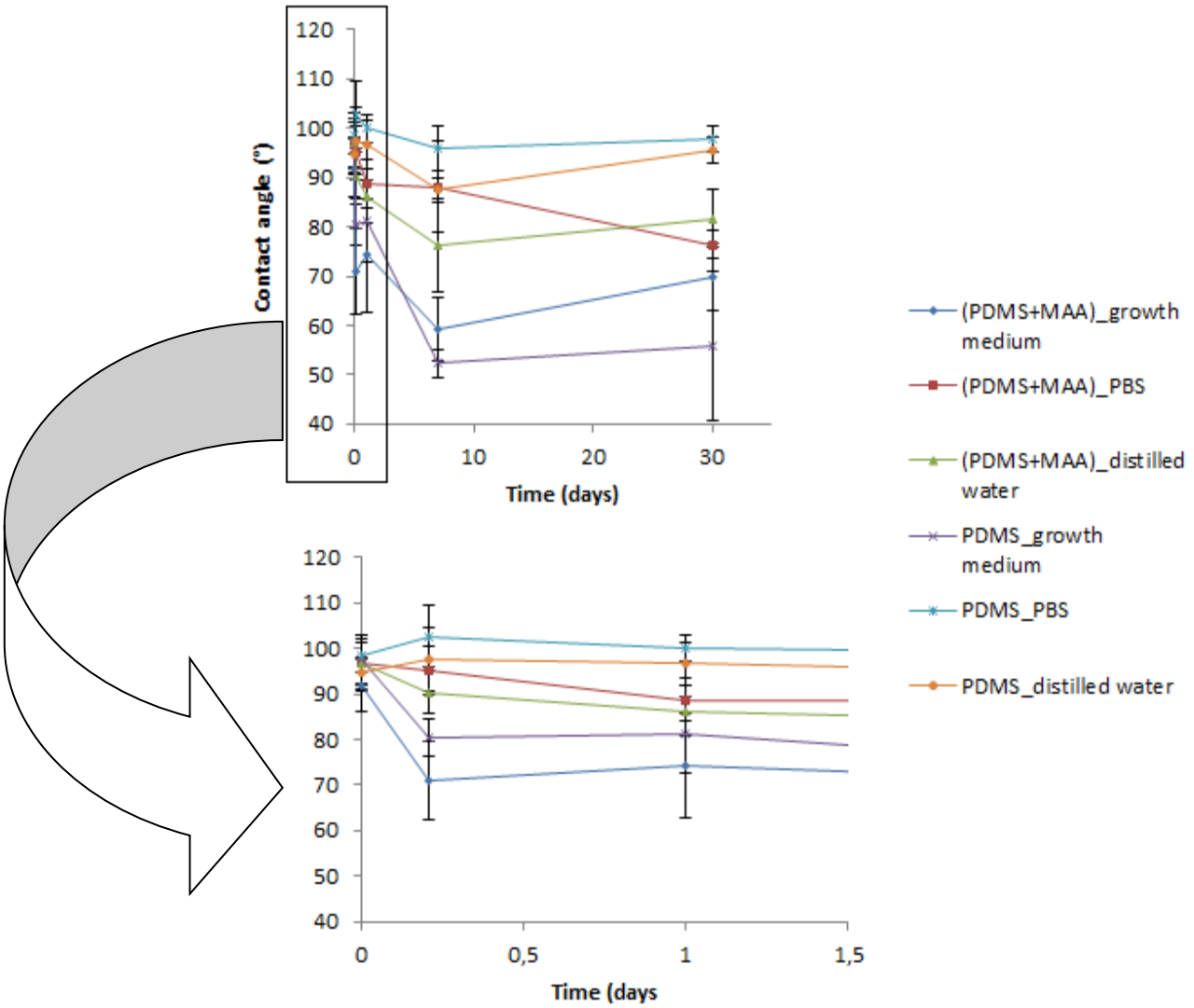

Figure 3.10 - Contact angle after UV modification of PDMS with MAA, under different storage mediums, along time

When was used MAA to modify the PDMS surface, the behavior of the samples when storage in different mediums, was similar to the HEMA grafted PDMS. Again, the lowest water contact angle is verified on PDMS unmodified, when stored in growth medium. Under distilled water and PBS, the surface modification was successful because in both cases, the modification by HEMA and MAA brought a decrease of the water contact angle, which is lower than the PDMS unmodified.

3.2.4 Cytotoxicity tests

The human fibroblasts cells were seeded at the same initial density in the 96-well plates, with or without materials to assess its cytotoxicity. Cell adhesion and proliferation in the presence of the materials was characterized through an inverted light microscope (Figure 3.11).

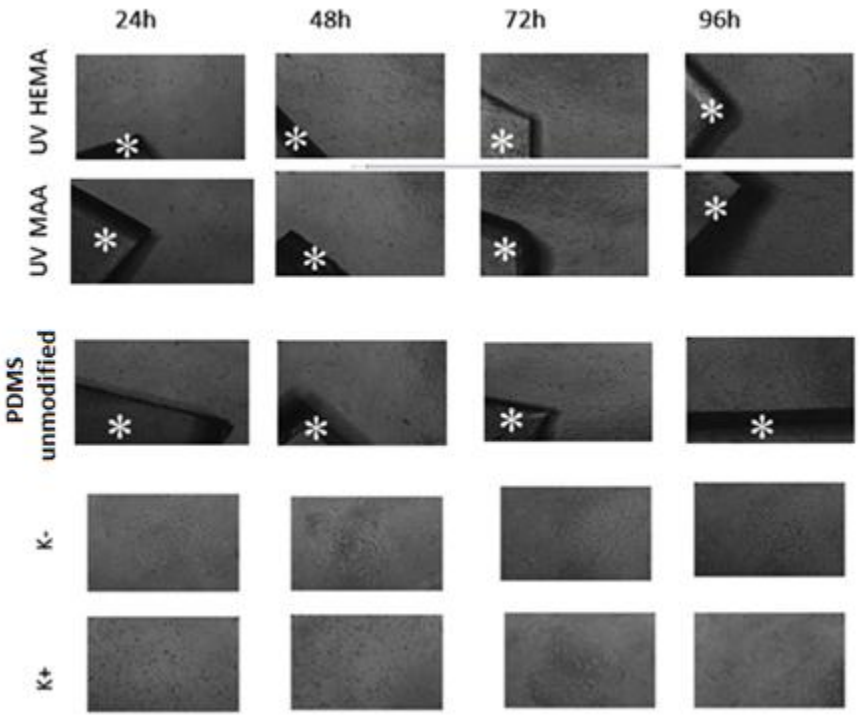

Figure 3.11 - Microscopic photographs of human fibroblasts cells seeded in the presence of the different PDMS materials (*) after 24, 48, 72 and 96h of incubation; K-, negative control; K+, positive control. Original magnification x100.

Figure 3.11 shows that cells adhered and proliferated in contact with all the materials and in the negative control. However, in the positive control no cell adhesion or proliferation was observed.

Furthermore, a MTS was also performed in order to further characterize materials cytotoxic profile. The MTS assay results (Figure 3.12) showed that cells in presence of tested samples had higher viability than in the positive control. After 4 days the cells remained viable, despite the end of this period the cells in contact with PDMS suffered a decrease on its viability. Moreover, along time, the cell viability was always greater for the modified materials than for the unmodified PDMS.

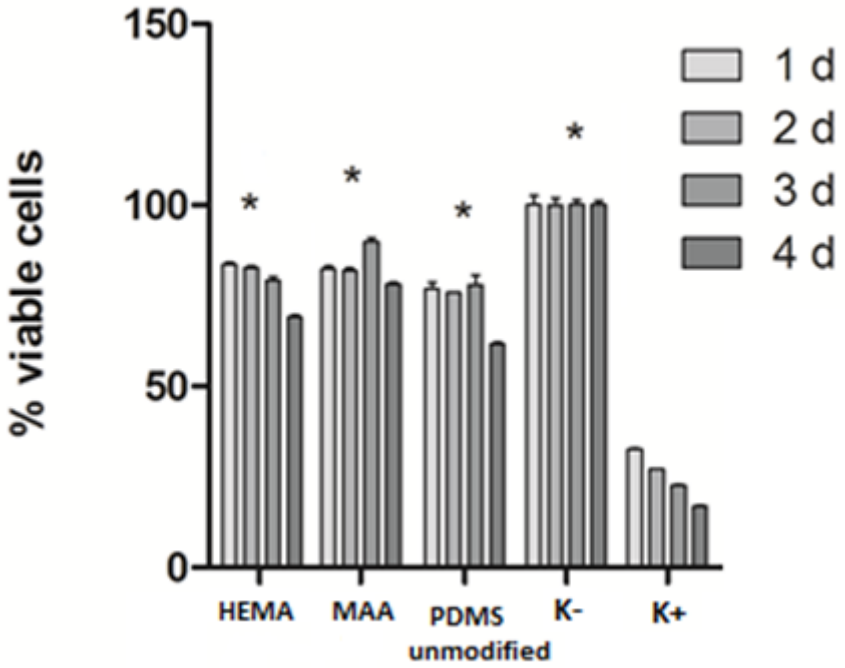

Figure 3.12 - Evaluation of the cellular activity of human fibroblasts cells seeded in the presence of the different PDMS materials after 24, 48, 72 and 96 h. MAA ; HEMA ; Positive control (K+); negative control (K-).Each result is the mean ± standard error of the mean of three independent experiments. Statistical analysis was performed using one-way ANOVA with Dunnet's post hoc test (*p < 0.001)

3.2.5 Antibacterial activity tests

In order to verify the affinity/non affinity of the materials tested for bacteria, were taken representative SEM micrographs across the material (Figure 3.13).

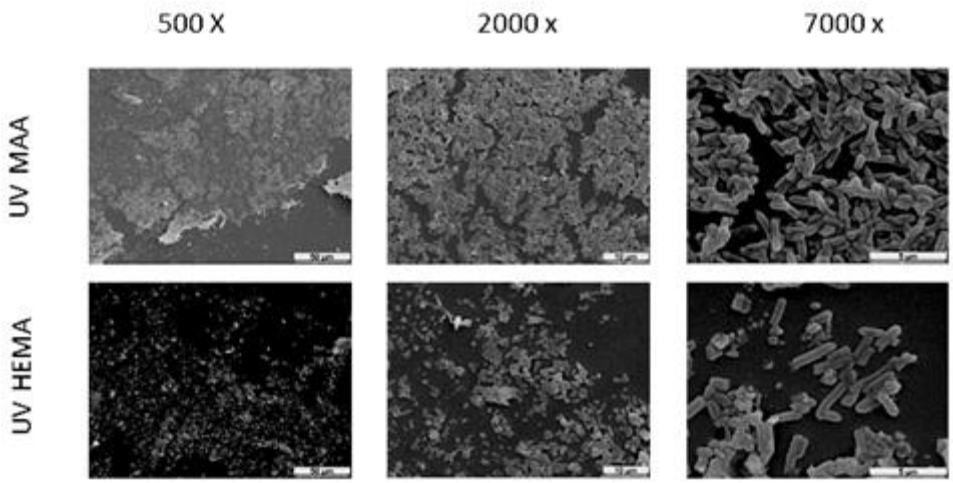

Figure 3.13 - SEM photographs of *E. Coli* seeded in the presence of the different PDMS materials at different magnifications 500 x, 2000 x and 7000 x.

Through this assay it is possible to observe the bacterial growth in all wells, which shows that these materials did not have an antibacterial effect. Nevertheless, through this SEM analysis it is possible to observe a reduction of the bacteria growth in the surface modification with HEMA when compared to MAA.

3.3 Discussion/ Conclusion

Surface modification by UV was not very effective in reducing the hydrophobic character of the surface (water contact angles remain high). However, there was a slight decrease of the water contact angle (approximately 10° for MAA and HEMA) from PDMS unmodified. Moreover, there was an increase of surface energy, which means that this technique was successful on grafting monomers with polar groups on PDMS surface.

The efficiency of storage of the films before and after the surface modification was distinct according to the medium used. This efficiency was evaluated by the hydrophobicity recovery of the PDMS films. When using the growth medium and PBS (on HEMA) there was a decrease of the hydrophilic behavior after 1 month, with PDMS films unmodified showing greater contact angles. In the same period, when distilled water and PBS were used (on MAA), as storage medium, the contact angle decreases compared with the unmodified PDMS.

The percentage of swelling was very low (less than 0.3%) which means that the PDMS films have no tendency to swell in the presence of fluids, which can be explained by the cross-linked structure and the highly hydrophobicity that this material exhibits.

The samples of PDMS, after modification with HEMA and MAA, were found to be non-toxic, a condition necessary for biomedical applications. However, when it was performed the characterization for determination of PDMS materials antibacterial activity, none of the samples showed antibacterial effect, which means that none of the samples inhibited bacteria growth. Moreover, the films grafted with HEMA showed a reduction of the bacteria growth in the surface.

4. Aminolysis on PDMS membrane

Recently, Aminolysis has been studied and developed to modify the surface of polymers like PCL (Polycaprolactone), PLLA (poly-L-lactide), PLGA (poly(lactic-*co*-glycolic acid)) and PDMS (Polydimethylsiloxane) In order to increase their biocompatibility and hydrophilicity. In this technique, the surface properties are modified without affecting the bulk of polymers. [128]

In this work, PDMS was aminolyzed to introduce amino groups on its surface.

4.1 Modification technique

In this technique, two different strategies were adopted.

First strategy

Films of PDMS were immersed in deionized water and dried under reduced pressure for 24h, at pressure 30ºC, until constant weight. Then, the PDMS films were immersed in a 1.6-hexanediamine/propanol solution with a concentration of 0.1 g/ml, at 37ºC for 24 and 48 hours. Then, the films were washed with deionized water at room temperature to remove the free 1.6-hexanediamine, and dried as previously.

Second strategy

Potassium hydroxide (0.48g) was dissolved in ethanol (5g). Afterwards, this solution was added to 9.8 g of 1.6 - hexanediamine to obtain a homogeneous solution. The amine solution and the PDMS film (2g) were placed in a glass vial, and continuously stirred at room temperature. Then, the films were washed with deionized water at room temperature, several times.

4.2 Results

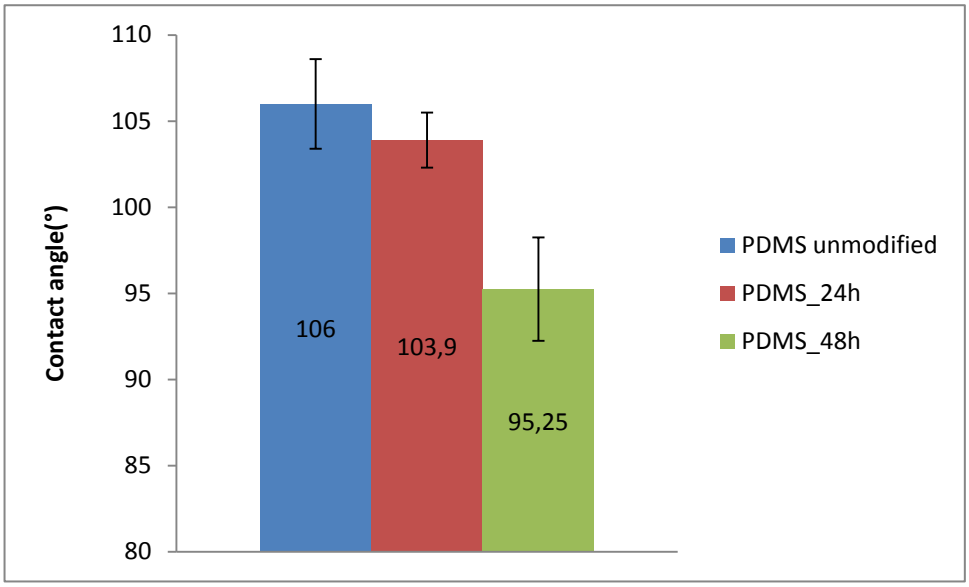

Figure 4.1 - Contact angle results of PDMS unmodified and when PDMS is modified with 1.6- hexanediamine in time of 24 h and 48 h. (First strategy)

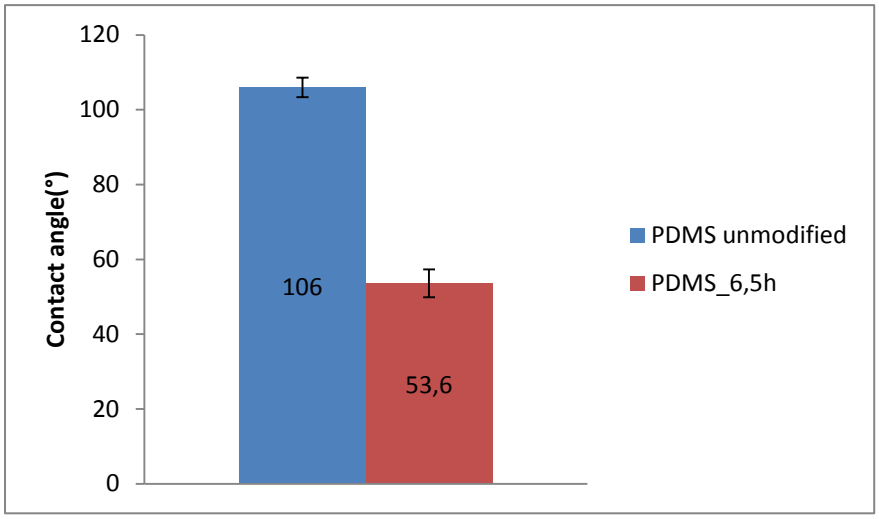

Figure 4.2 - Contact angle result of PDMS unmodified and when PDMS is modified in time of 6.5 hours. (Second strategy)

Figure 4.1 and 4.2 show the contact angle when the amine groups were introduced in the PDMS surface, with the first and second strategy respectively.

Although there is a clear reduction in the water contact angle in the figure 4.1 (≈2° for 24h and ≈11° for 48h from PDMS unmodified), using the first strategy, the second one proves to be better on reducing the contact angle (≈52°). Therefore, the

first strategy was abandoned. The next methods presented will only consider the second strategy.

In this method, KOH was used as the catalyst and ethanol ensures the completely dissolution of hydroxide potassium and increases the cleavage reaction rate. The reaction between 1.6-hexanediamine and PDMS results on the grafting of the -NH_2 groups onto PDMS surface as schematically represented in figure 4.3.

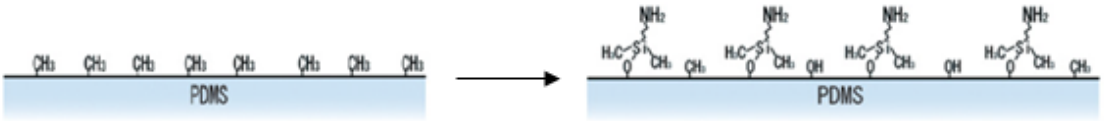

Figure 4.3 - Grafting procedure of –NH_2 groups on the PDMS surface, after modification with 1.6 - hexanediamine

The structure of PDMS films after modification were identified and analyzed by FTIR. For a better understanding of which groups were introduced on the PDMS surface, a superposition of the FTIR spectra of PDMS surface after modification with FTIR spectra of PDMS unmodified and 1.6-hexanediamine is illustrated in figure 4.4. As expected unmodified PDMS films do not show amine groups (1640-1500 cm^{-1} for N-H bend and 3500-3300 cm^{-1} for N-H stretch) on its structure contrarily to PDMS after modification with 1.6-hexanediamine. The introduction of –NH_2 groups into the surface of PDMS, brought an increase of the polar component, which can be seen in the figure 4.5.

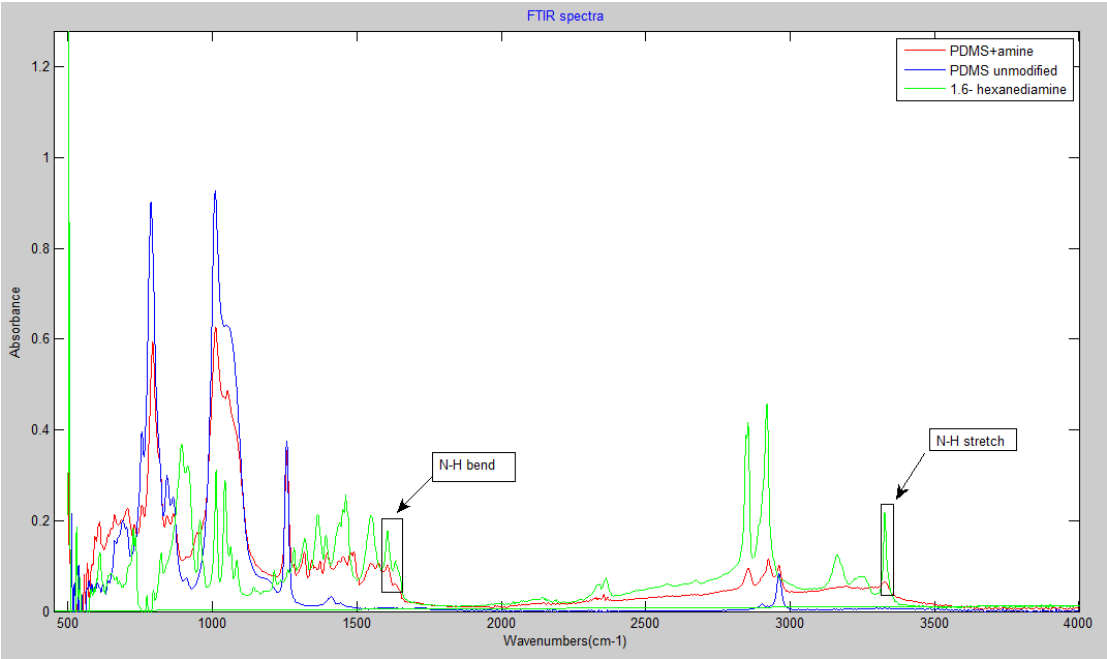

Figure 4.4 - FTIR spectra of PDMS unmodified, 1.6 - hexanediamine and PDMS modified with 1.6 – hexanediamine

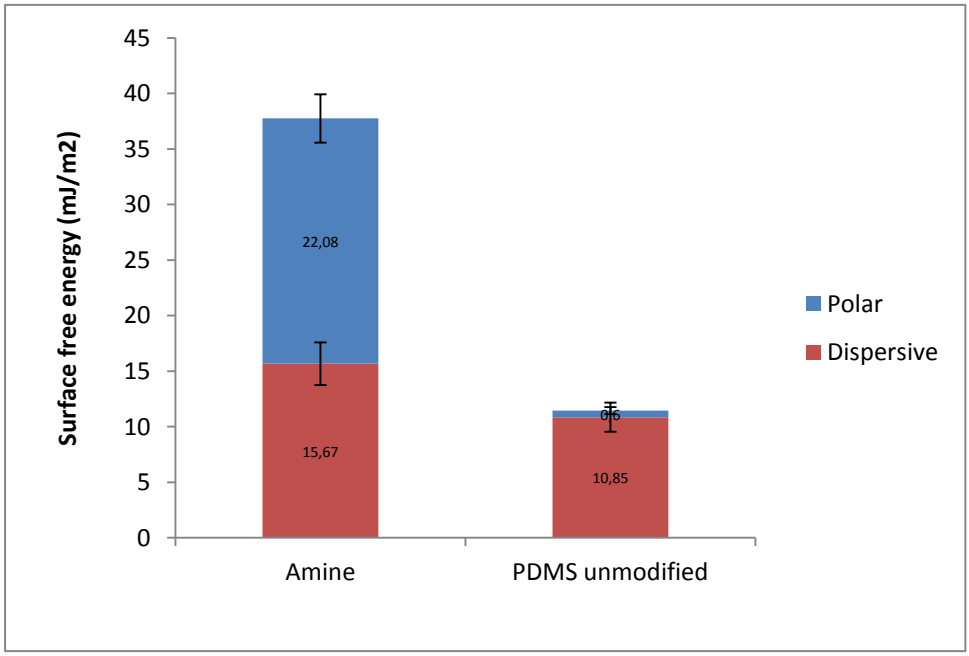

Figure 4.5 - Surface free energy of PDMS unmodified and pdms modified with 1.6 - hexanediamine.

4.2.1 Swelling/Degradation

Figure 4.6 shows the behavior of aminolysis of PDMS films over time. There was a slight decrease of weight of pdms films probably due to the interaction of amine groups with the storing samples.

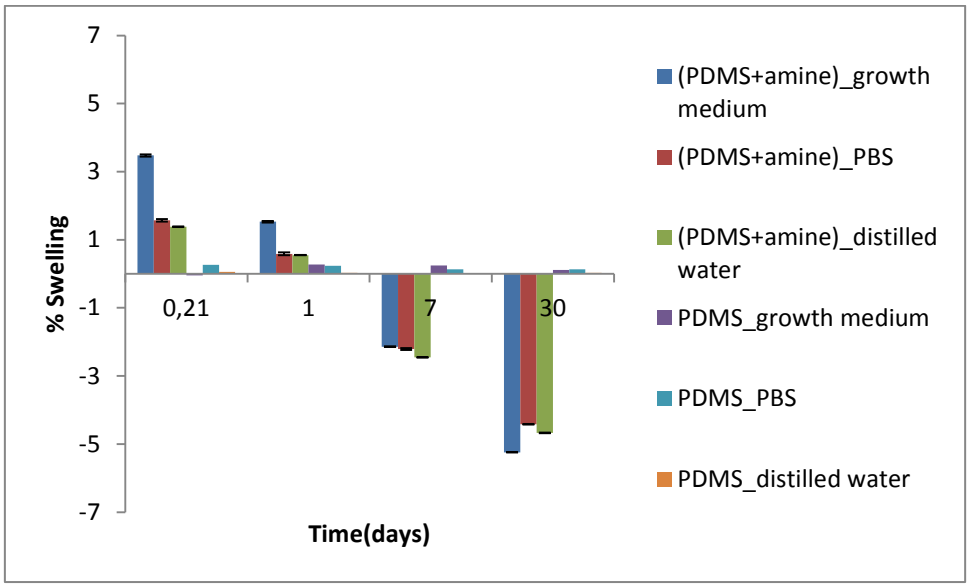

Figure 4.6 - Percentage of swelling after the modification of PDMS by 1.6 - hexanediamine, under different storage mediums, along time

The decrease of the amine groups on PDMS surface due to the interaction with the storage medium can be seen in the figure 4.7, which shows the hydrophobic recovery over time. After 1 week, there was a significant increase of the contact angles in the three samples, corresponding to the reduction of the percentage of swelling observed in figure 4.6 to one week.

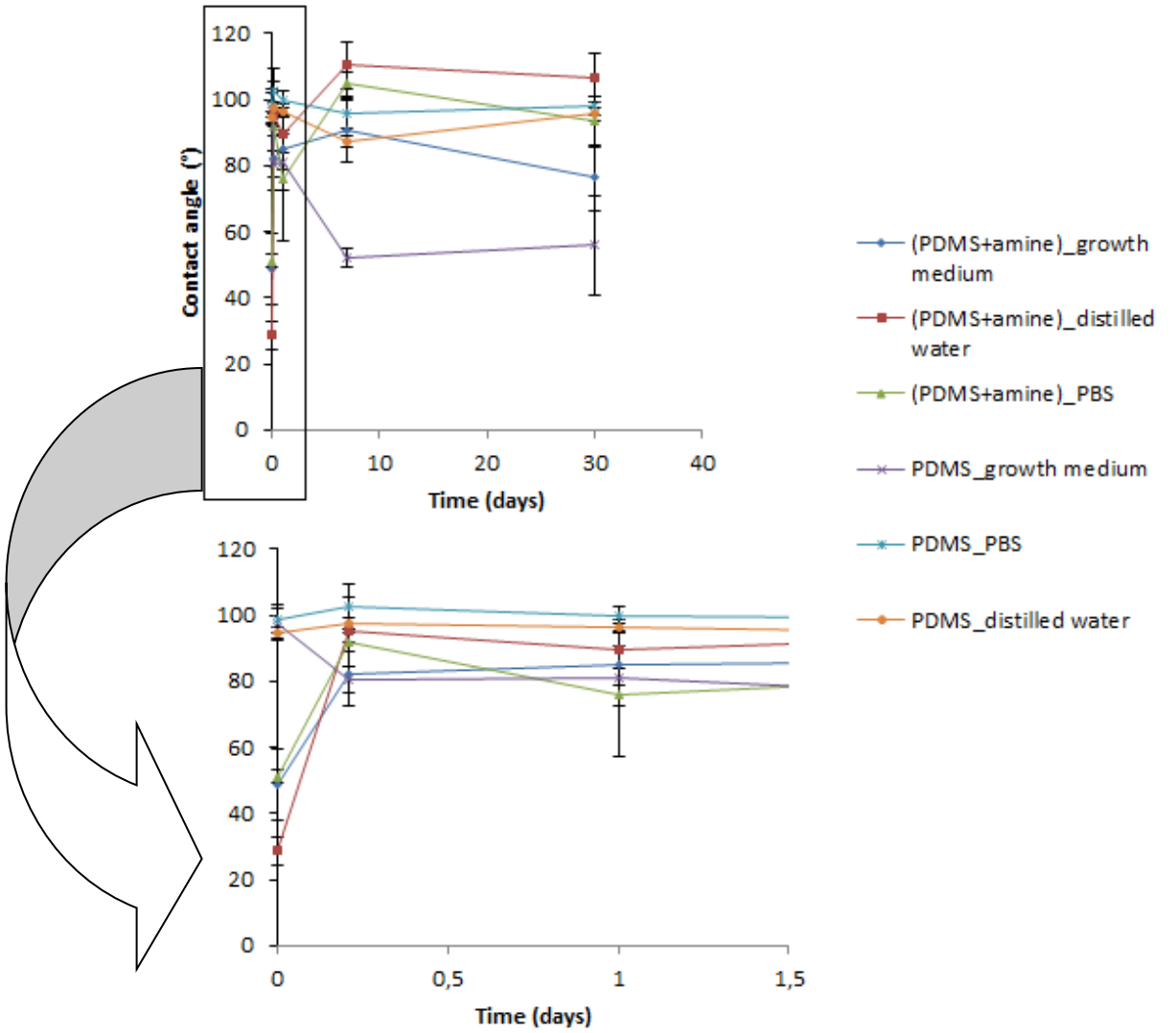

Figure 4.7 - Contact angle after modification of PDMS by 1.6 - hexanediamine, under different storage mediums, along time

4.2.2 Cytotoxicity tests

The human fibroblasts cells were seeded at the same initial density in the 96-well plates, with or without materials to assess its cytotoxicity. Cell adhesion and proliferation in the presence of the materials was characterized through an inverted light microscope (Figure 4.8).

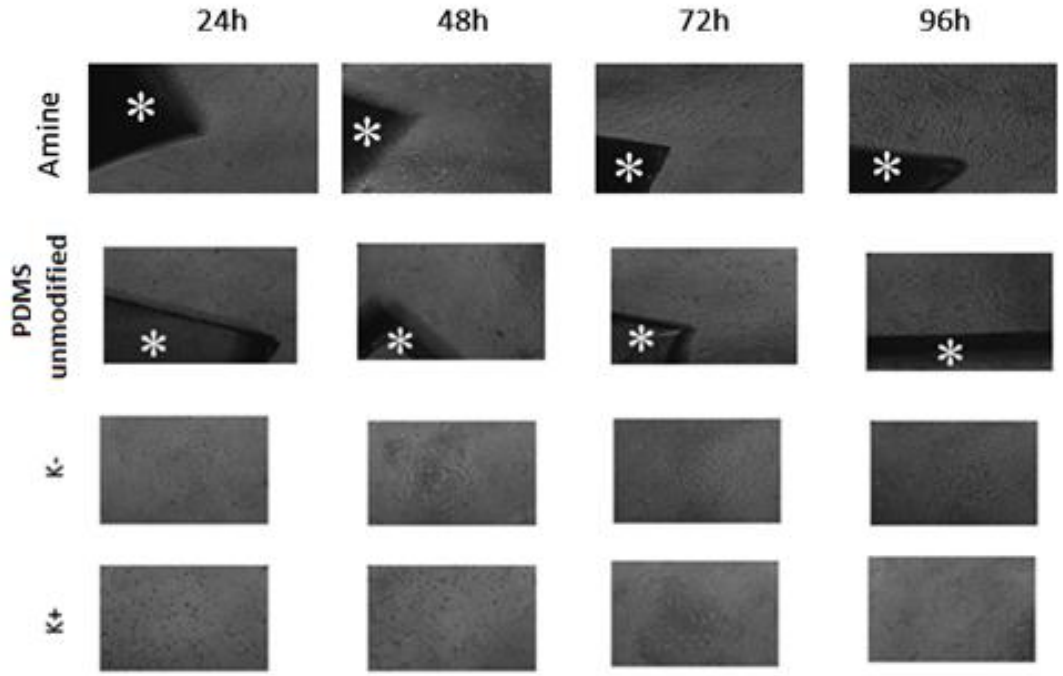

Figure 4.8 - Microscopic photographs of human fibroblasts cells seeded in the presence of the different PDMS materials (*) after 24, 48, 72 and 96h of incubation; K-, negative control; K+, positive control. Original magnification x100.

Figure 4.8 shows that cells adhered and proliferated in contact with all the materials and in the negative control. However, in the positive control no cell adhesion or proliferation was observed.

Furthermore, a MTS assay was also performed in order to further characterize materials cytotoxic profile. The MTS assay results (Figure 4.9) showed that cells in presence of tested samples had higher viability than in the positive control. After 4 days the cells remained viable, despite the end of this period the cells in contact with PDMS, suffered a decrease on its viability. Moreover, along time, the cell viability was always greater for the modified materials than for the unmodified PDMS.

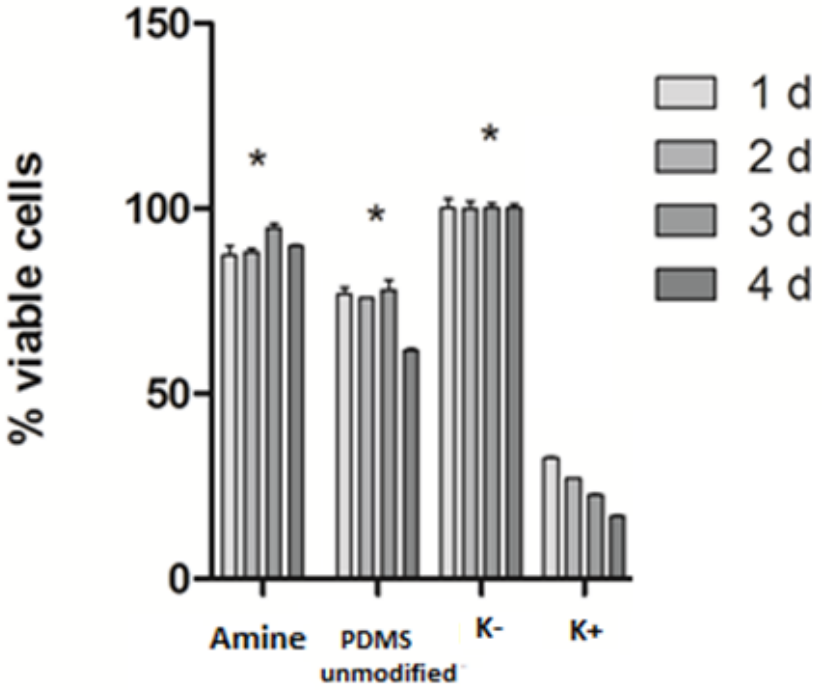

Figure 4.9 - Evaluation of the cellular activity of human fibroblasts cells seeded in the presence of the different PDMS materials after 24, 48, 72 and 96 h Amine; Positive control (K+); negative control (K-).Each result is the mean ± standard error of the mean of three independent experiments. Statistical analysis was performed using one-way ANOVA with Dunnet's post hoc test (*p < 0.001)

4.2.3 Antibacterial activity tests

In order to verify the affinity/non affinity of the materials tested for bacteria, were taken representative SEM micrographs across the material (Figure 4.10).

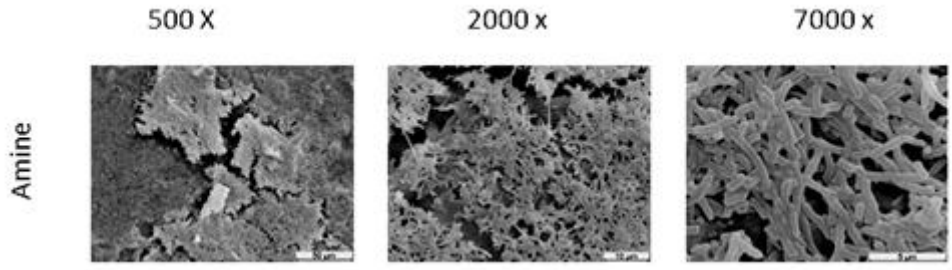

Figure 4.10 - SEM photographs of *E. Coli* seeded in the presence of the different PDMS materials at different magnifications 500 x, 2000 x and 7000 x.

Through this SEM analysis is possible to observe the bacteria growth in the surface, which shows that this material does not have an antibacterial effect, i.e. do not avoid biofilm formation.

4.3 Discussion/ Conclusion

The second strategy (when PDMS is modified in time of 6.5 hours) proved to be more effective than the first strategy on reducing the water contact angle.

Surface modification of PDMS by amine was very successful in reducing the hydrophobic character of the surface, which can be explained by the introduction of amine groups on surface, leading to an increase of the polar component of surface free energy.

The main problem of this technique was the maintenance of the hydrophilic character of the surface over time. After 1 week, there was a considerably increase of the water contact angle for the three storage mediums. After this, the contact angle slightly decreased, although to values still remained high, and they were higher than the unmodified PDMS.

The sample of PDMS, after modification with 1.6 - hexanediamine, was found to be non-toxic, a property that is fundamental for biomedical applications. Moreover, the microbiological studies revealed that the PDMS materials did not have antibacterial effect, allowing bacterial growth.

5. Plasma surface Treatment

When energy is continuously applied to matter, this suffers a process from a solid to liquid and gas, caused by the increasing temperature. Carrying on applying energy causes the breaking up of the atom. This mixture of radicals, negatively charged electrons and positively charged ions, is called plasma. [101] It is naturally referred to as the fourth state of matte, because 99% of the universe known to man is in the state of plasma. [102]

Plasma can be found, in nature, in lightning, flames and in the sun. Among other things, artificially created plasma is found in plasma televisions, neon lights and flashback lights. [103]

As opposed to an ordinary gas, free electrical charges in plasma lead to high electrical conductivity that could even exceed those of metals. [104]

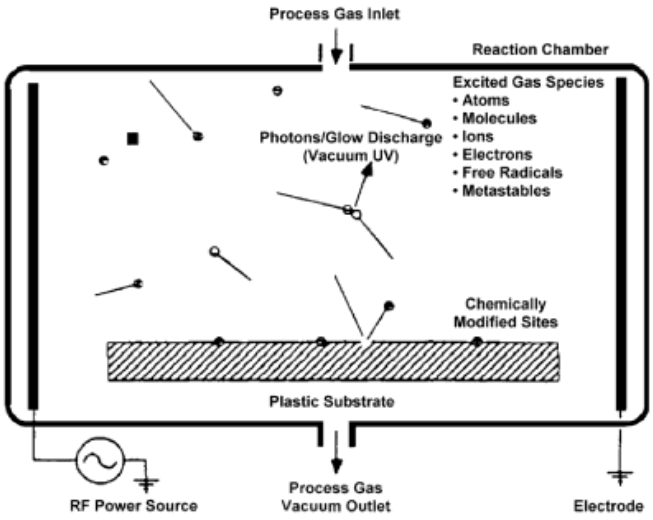

Figure 5.1 - Schematic figure of the surface modification of plastic in a gas-plasma reactor [104]

The gaseous system is highly reactive and can activate inert surfaces, enabling the application of metallic coatings, ceramic and polymer in a variety of materials. The plasma might serve to crosslink, cleaning, oxidize or to introduce functional groups on the material surface. [105], [106]

There are some advantages of the plasma-based techniques [107]:

1- Sterile surfaces might be provided by plasma processing. It can be scaled up to industrial production reasonably easily.
2- Typically, plasma engineering is reproducible, reliable, fairly inexpensive, non-line-of-sight, applicable to different materials like composites, metals, ceramics and polymers and applicable to diverse sample geometries.
3- Advantages of plasma treatment came from a good perception of plasma chemistry and physics, such as plasma homogeneity and effects of non-uniform plasma on the substrate surface.
4- Masking techniques are compatible with plasma treatment to allow surface patterning, which is regularly applied in the microelectronics industry.
5- Plasma processing could result in alteration of a diversity of surface characteristics.

The plasma processing of materials is increasing significantly, covering many activities like deposition, coating technologies and the manufacture of electronic materials. [108]

Plasma process could be grouped in two different classes: nonthermal plasmas and thermal plasmas. The non-thermal plasma, also referred to as “nonequilibrium plasma”, “low-temperature plasma”, “cold plasma” or “non-isothermal plasma” is featured as different energy states between particles in the plasma. The temperature, in the non-thermal plasma, is not in thermal equilibrium and differs considerably between the electrons and the other particles (ions, atoms and molecules). Electrons have a small mass, and because of that, they can be easily accelerated under the control of an electric field. The temperature of electrons normally ranges from 10000K to 250000K. The production of free radicals is made by these highly energetic electrons from parent molecules by several steps of chemical and physical processes. Typically, nonthermal plasma is operated at room temperature and atmospheric pressure. Thermal plasma, also named as “thermal equilibrium plasma” or “hot plasma”, might be used for the treatment of liquid waste, solid waste and waste gas of high concentration. Temperature in the thermal plasma, reaches around 10273 K, which all its components are at thermal equilibrium. [109]

Depending on the desired functionalisation, different gases are used: for Hydrophilic proprieties is used O_2, N_2, NH_3, H_2/N_2, Ar and for adhesion, the gases used, are O_2, N_2,NH_3, Ar/N_2, Ar, CO_2, $Ar/O_2/NH_3$,H_2. [110]

Following plasma treatment, the wettability of polymers changes potentially due to unsaturation effects, electrostatic charging, oxidation and surface morphology changes. In biomaterials surface, one of the main interests, is the improvement of surface wettability by the fact that most common polymeric biologics are hydrophobic in nature, such as PTFE, PE, PP, PMMA, PS, PET, PVC, polyurethane and silicone rubber. Hydrophilic surfaces can be achieved by treating polymers with nitrogen, oxygen or water plasma. [111] Plasmas of NH_3 and N_2 are used to generate amine groups on the surface of PTFE and PS, respectively, while the inert gases may be used to create radicals in the polymer surface and then performing a vinyl polymerization. [112]

The commonly-desired reactive centers which can be generated by plasma treatment are primary amine groups, hydroxyl and carboxylic, as shown in table 5.1 [111]

Table 5.1 - Effects of plasma treatment on polymer surface modification [111]

Effect	Surface Change	Plasma Gas
Wettability	**Oxidation, electrostatic unsaturation**	**O_2, N_2, H_2O, air(inert gases), NH_3, CO_2**
Molecular weight	**Crosslinking**	**Inert gases (He, Ar), H_2, N_2**
	Degradation	**O_2, N_2**
	Etching	**Ar, CF_4**
Functionalization (reactive sites)	**-OH**	**O_2,H_2O, H_2O/H_2O_2**
	-C(O)OH	**CO_2**
	-C-O-O-	**Ar (quenching in O2 or air)**
	-NH_2	**$N_2 + H_2$, NH_3**
	C=C	**Inert gases**

The controlled removal of a desired material from a substrate through physicochemical methods is called etching. It could be executed using chemicals in gaseous or plasma phase in liquid state - dry etching (includes ion milling, gas phase chemical etching, chemically assisted ion beam etching, reactive ion etching and

plasma etching) or could be executed using chemicals in liquid state - wet etching. [114]

In some cases, a phase of the material could be more susceptible to treatment by plasma, which results in a marked surface with the chemistry of the resistant material to plasma. [25]

Plasma on silicone materials

In 1970, Hollahan and Carlson discovered CH_2OH groups in the modified-surface of PDMS, which was treated with oxygen plasma and corona discharge using Fourier Transform Infrared Spectroscopy (FTIR) characterization. In 1986, Bodo and Sundgren show the effectiveness of the PDMS modified surface. [115]

On exposure to oxygen plasma, PDMS materials could acquire silanol groups at the expense of methyl groups (elimination of $-CH_3$ groups) or methylol groups (no elimination of $-CH_3$ groups), as illustrated in figure 5.2. In the first case, also free radicals (O•) can be formed and then converted to peroxides (ROOH). The oxidation of the surface layer augments the concentration of hydroxyl groups, which leads to the formation of strong intermolecular bonds. Because of the polar nature of silanol groups, the exposition of these makes the surface of PDMS highly hydrophilic. [116] ,[117]

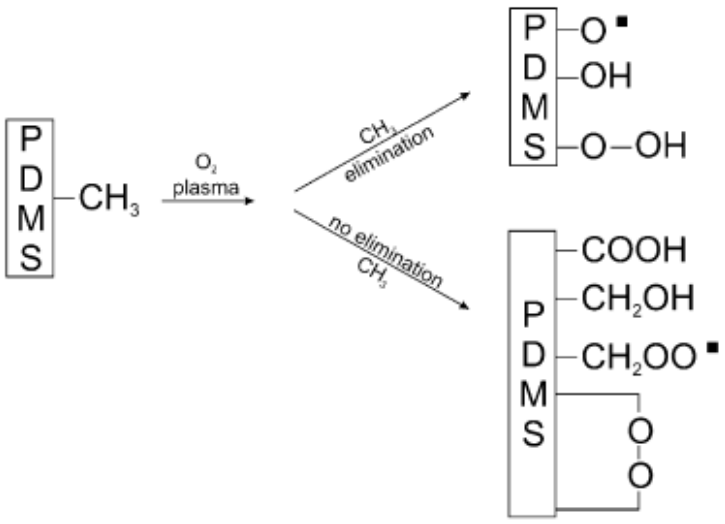

Figure 5.2 - Two possible reactions for PDMS surface activation with oxygen plasma.

By contrast, when PDMS materials are exposed to inert gases such argon, plasma processing cannot by itself bring in functional groups on the polymer surface. It has been expected that Noble gases like Argon might be used to produce free radicals at the polymer surface, by breaking, in the polymer substrate, C-H or C-C, as illustrated in figure 5.3 [118]

However, the plasma technique has a disadvantage oh having a short lifetime due to the hydrophobic recovery. By chemical or physical-chemical methods, the hydrophilicity on PDMS surface can be prolonged. This prolongation requires complex protocols and it's very expensive.[119]

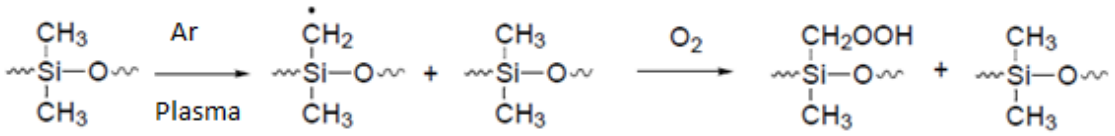

Figure 5.3 - One possible reaction for PDMS surface activation with argon plasma. [91]

The hydrophobic recovery of oxygen plasma treated PDMS must not only be assigned to the diffusion of low molecular weight (LMW) chains from the bulk to surface but also to the elastomeric proprieties of PDMS materials, which mechanically recovers back after ion bombardment. The morphology of nanostructuring of the film surface is, as well, a strong cause behind the low hydrophilicity and also hydrophobic recovery of PDMS. [105] Frequently, plasma treatment could result in a number of different features with a low stability. It is wanted, therefore, to reduce or, if possible, to avoid this effects. Two kinds of strategies are applied. . The first is minimizing the kind and density of harmful particles over treatment in the plasma and minimizing the used energy. The second is separating substrate functionalisation from plasma in time (grafting) or in space. These methods contribute to a more homogeneous distribution of functionalities and a better preservation of the precursor structure. [120]

5.1 Modification technique

The modification with plasma was performed with a small-scale plasma system (Figure 5.4). For this procedure were used 2 gases, oxygen and argon. The influence of time and pressure with both gases was evaluated.

Figure 5.4 - Plasma equipment used in the surface modification of Silicone based Materials.

5.2 Results

Figures 5.5 and 5.6 show the results of the contact angle of the PDMS surface when exposed to a different time and pressure, for argon and oxygen respectively.

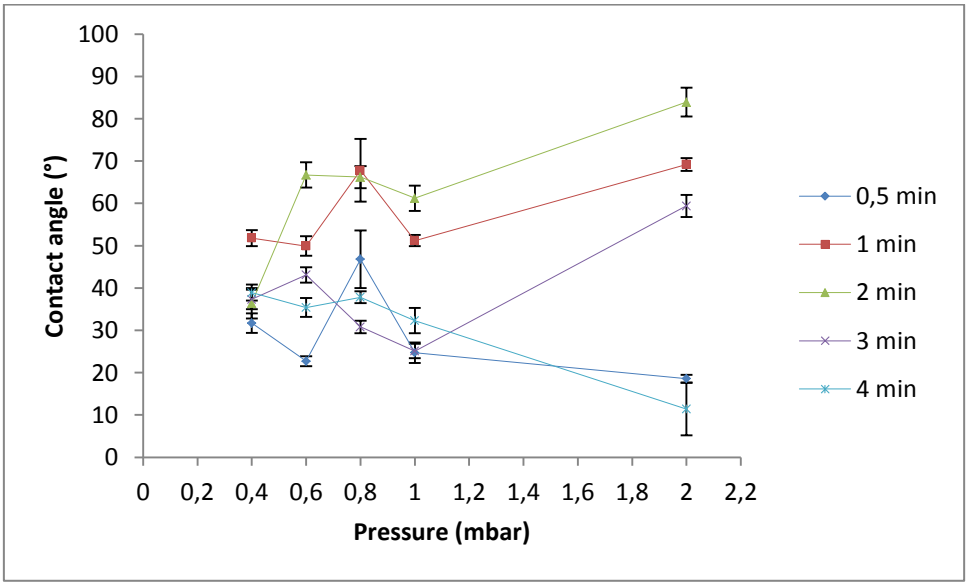

Figure 5.5 – Water Contact angles, by varying the pressure and the time of processing, using oxygen as working gas

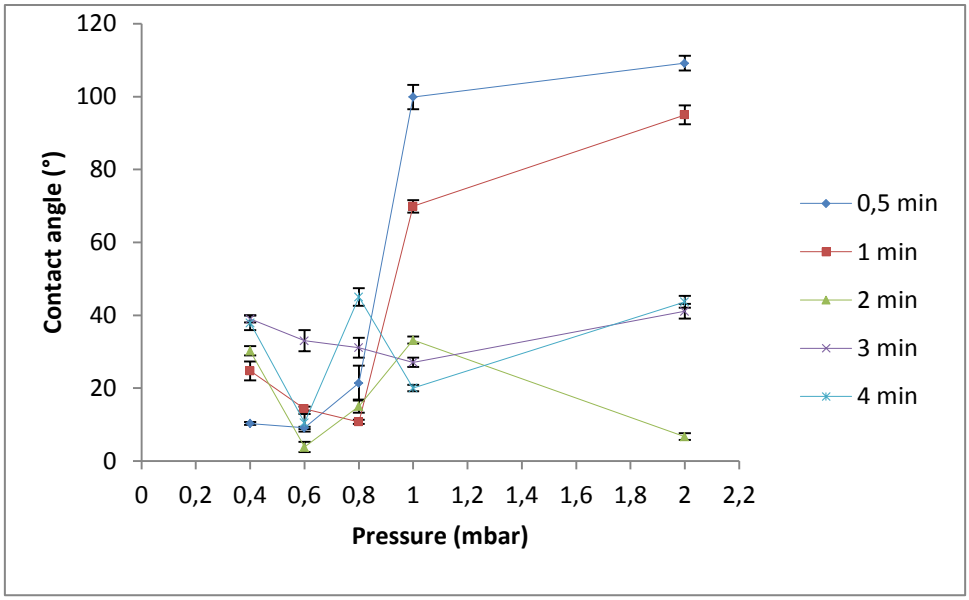

Figure 5.6 – Water Contact angles, by varying the pressure and the time of processing, using argon as working gas

The results obtained revealed a significant decrease in contact angles using argon and oxygen as working gases. The result that leads to the lowest water contact angle were obtained when argon was used as working gas, at a pressure of 0.6 mbar, for 2 minutes (decrease of ≈102° from PDMS unmodified).

5.3 Discussion/ Conclusion

Argon and oxygen have different mechanisms (regarding to the surface modification), when are in contact with the PDMS surface. In this work, it was obtained better hydrophilicity when argon was used as working gas.

When oxygen is used, as working gas, there is a substitution of the methyl groups by silanol groups (caused by the linkage between gas radicals and radical polymer chains), argon favors the formation of polymer radicals (no formation of gas radicals because argon is a noble gas). The formed radicals will then react with the atmospheric air leading to Si-OH and Si-CH_2OH groups on the surface of PDMS.

The main problem of plasma-treated surface is its instability along the time, called “hydrophobic recovery”, as previously mentioned. This process could be attenuated or even eliminated by the grafting of the surface with a vinyl monomer,

which maintains the hydrophilicity and improves the free functional groups available at its surface.

This process was very important, because despite not being a definitive method for modifying the surface of PDMS, provided the optimal information of processing, using oxygen and argon as working gases.

6. Plasma-induced graft polymerization of Methacrylic acid (MAA) and 2-hydroxyethylmethacrylate (HEMA) on Poly(dimethylsiloxane) Surfaces

Normally, for surface modification, plasma-graft polymerization has been used to introduce hydrophilic groups. The monomers chosen for graft copolymerization should be vinyl compounds with a high rate of propagation. In this procedure, firstly a polymer specimen is exposed to a suitable plasma like argon or oxygen, and then comes into contact in the aqueous or organic solution of a monomer, at a high temperature, for a long period. (Polymerization reaction due to radicals obtained in the plasma media). [121]

The durability of the modified surface is the main advantage of plasma graft polymerization over the plasma surface treatment. The properties originated by plasma surface treatments repeatedly suffer from the recession with aging, while the surface characteristics enhanced by the graft plasma polymerization do not modify easily. [122]

At a high temperature, the graft polymerization of hydrophilic monomers like the 2-hydroxy-ethylmethacrylate (HEMA) and Methacrylic acid (MAA) could be initiated by the decomposition of peroxides, according to figure 6.1.

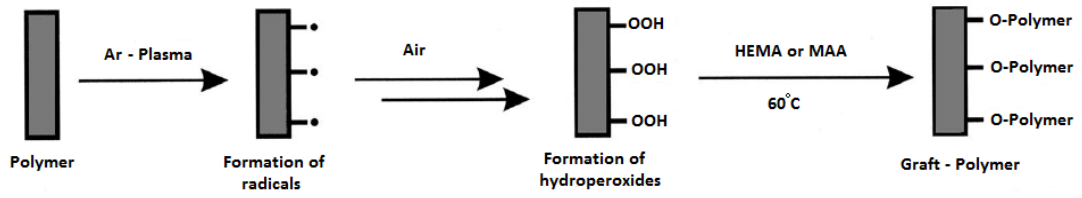

Figure 6.1 - Schematic illustration of plasma-induced graft copolymerization of polymer surfaces

After the best conditions found previously, as regards to time and pressure for argon and oxygen (pressure of 0.6 mbar, for 2 minutes using argon as working gas), it was studied the most accurate time for graft polymerization on pdms surface by HEMA and MAA.

6.1 Modification technique

At a chamber pressure of 0.6 mbar, for 2 min, PDMS films were plasma treated. Then, the films were dipped into a 10% (v/v) aqueous solution of HEMA or MMA and were placed in an oven at 60°C, for different times (30min, 1h, 2h, 4h, 6h 8h and 24h). Then, PDMS films were narrowly washed with water and dried until constant weight.

6.2 Results

Figure 6.2 shows the water contact angle results of plasma-induced graft polymerization HEMA and MAA on PDMS surfaces, at different times.

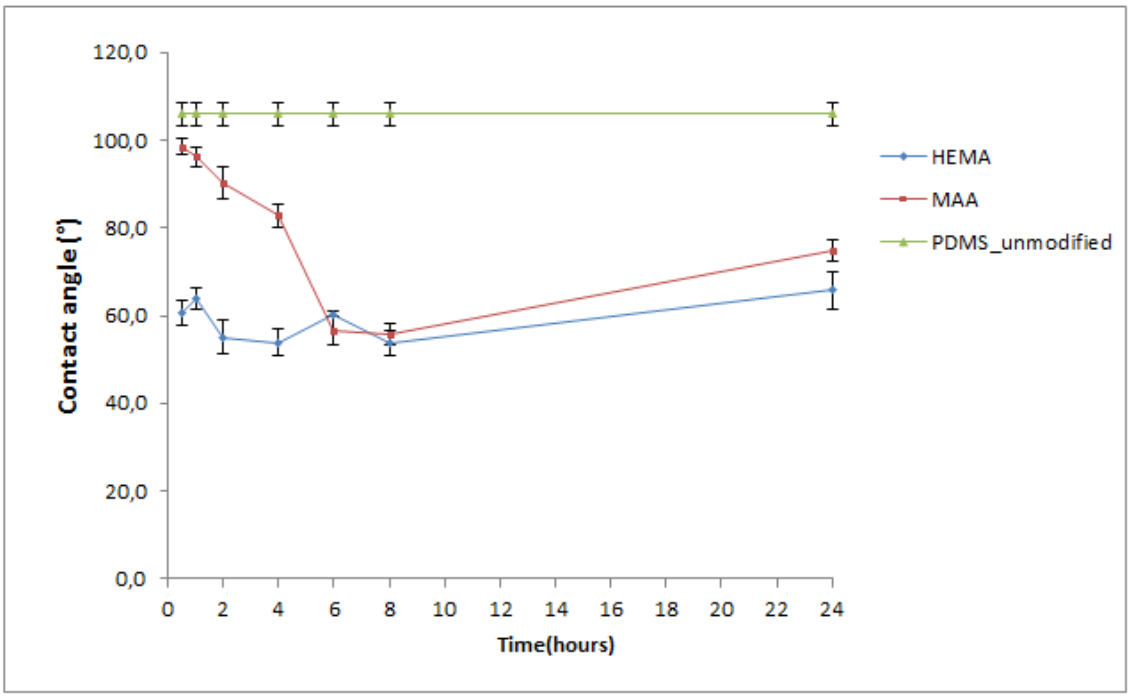

Figure 6.2 - Contact angle values of Plasma-induced graft polymerization of HEMA and MAA, along time

The lowest water contact angles results were obtained at 8 hours, for both HEMA and MAA. However, the behavior of the two monomers after grafting was different. While with HEMA, the variation of water contact angles were low (±10°), the variation of water contact angles were higher when MAA was used (from 98.5° at 30 minutes to 55.7° at 8 hours), which means that, in this monomer its necessary 6-8

hours of thermal aging for crosslinking or removal of low molecular weight species present in the bulk. (which prevents the migration of LMW PDMS chains to the surface to cover up the thermodynamically unstable hydrophilic surface).

After the 8 hours, there is an increase of the contact angle, for both monomers, which means that thermal aging is no longer able to delay the hydrophobic recovery of argon plasma activated surfaces.

With the best conditions for graft polymerization of HEMA and MAA on PDMS surfaces determined, the next step was to evaluate the surface free energy, the analysis of swelling / degradation of the films in different ways and the hydrophobicity recovery. Finally the antibacterial activities of the PDMS films and cytotoxic profile of the materials was also characterized.

6.2.1 Surface free energy

The presence of polar groups such as SiO_2, Si–OH and Si–CH_2OH at the surface brought an increase of the polar component on PDMS surface, as illustrated in figure 6.3.

As shown by the contact angle HEMA reacts better to thermal aging after plasma treatment than MAA. Contrary to the UV technique the polar component of HEMA is much higher, which can be explained by the fact that the OH groups are not wrapped in methyl groups, but pointed outward.

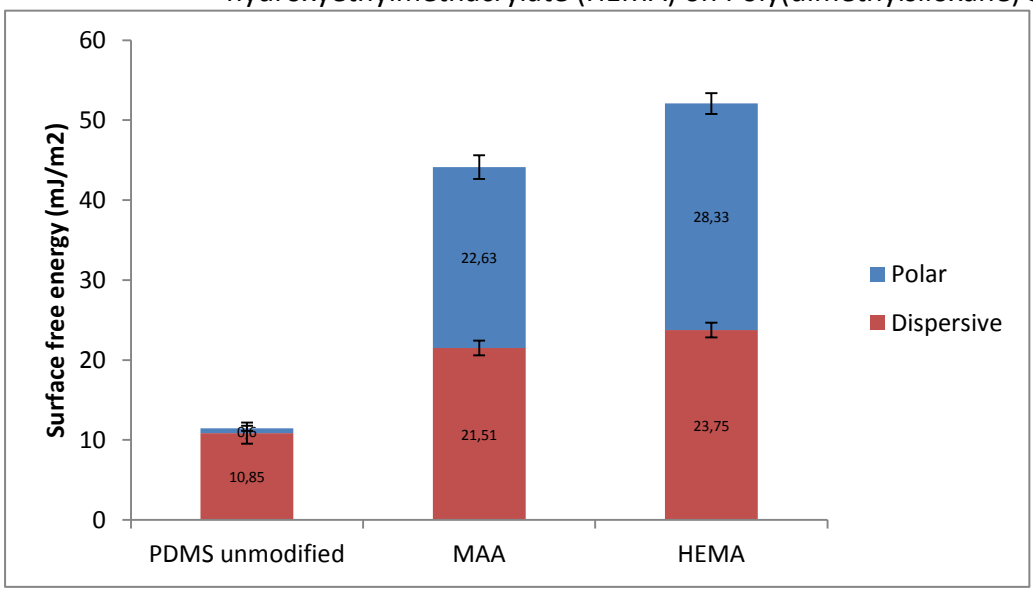

Figure 6.3 - Surface free energy of the unmodified and modified PDMS (PDMS argon plasma activated coated with HEMA and MAA)

6.2.2 Swelling/Degradation

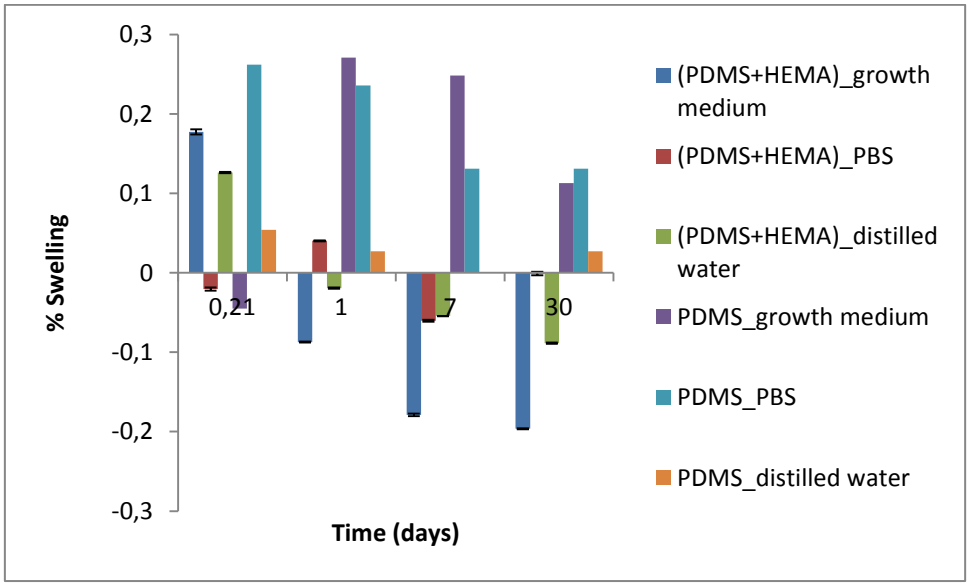

Figure 6.4 - Percentage of swelling of Plasma-induced graft copolymerization of HEMA onto PDMS surface, under different storage mediums, along time

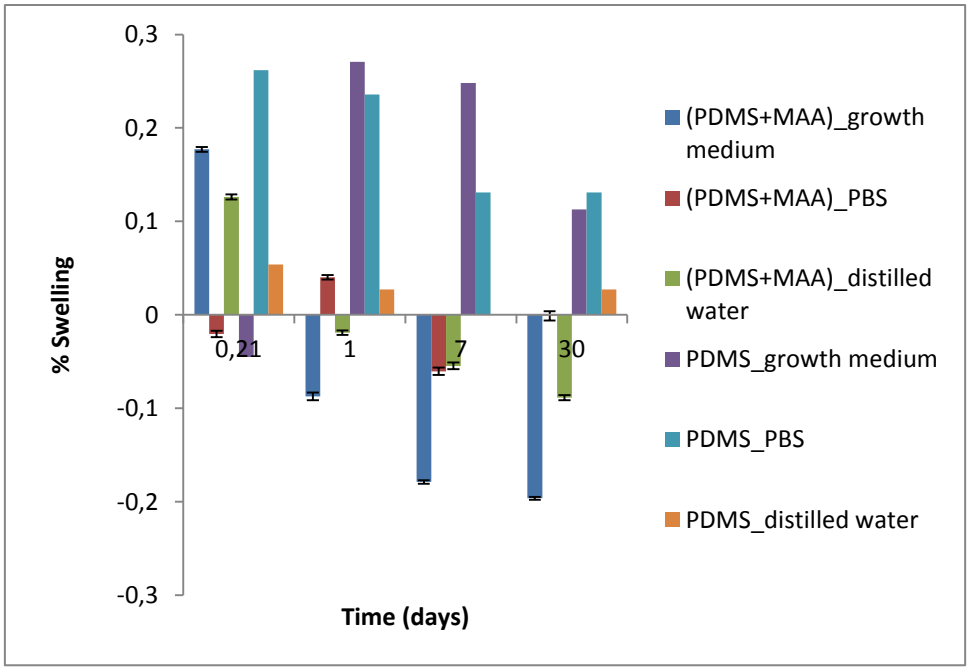

Figure 6.5 - Percentage of swelling of Plasma-induced graft copolymerization of MAA onto PDMS surface, under different storage mediums, along time

As observed in figures 6.4 and 6.5, the percentage of swelling/degradation was almost zero, which means that after modification PDMS silicon films remains highly reticulated and therefore the percentage of swelling/degradation is very low. Nonetheless, after one day, under the three storage mediums, modified PDMS films started to lose some weight (0,1%-0,2%), especially under growth medium, contrary to unmodified PDMS. This loss of weight could be confirmed in hydrophobic analysis, as illustrated in figures 6.6 and 6.7, where there was a slight increase after the first 5 hours.

6.2.3 Hydrophobic recovery

After one month, the three storage mediums (distilled water, PBS and growth medium) used proved to be able to keep the hydrophilic behavior of the PDMS surface, for both monomers (HEMA and MAA). This modification technique brought a decrease of the water contact angle when compared to PDMS unmodified under distilled water, PBS and growth medium. However, when using PBS and distilled water, as storage medium, the fluctuation of the contact angles was almost constant, with slight ups and downs. When using growth medium the fluctuations of the water contact angle were more accentuated, registering a marked loss in the first five hours

for both monomers, which can be explained by the introduction of amino and carboxyl groups on the surface of PDMS, from proteins present in growth medium.

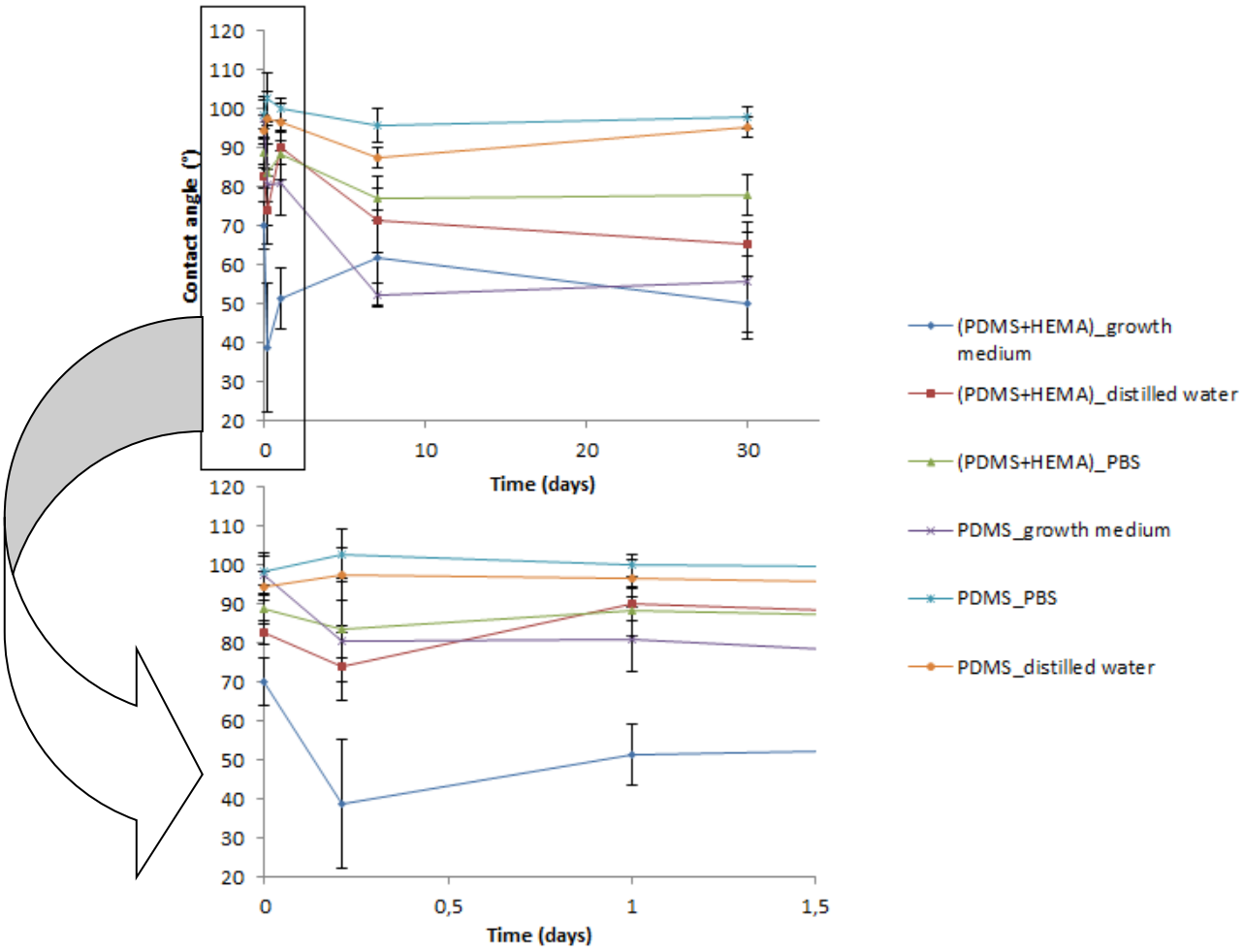

Figure 6.6 - Contact angle of Plasma-induced graft copolymerization of HEMA onto PDMS surface, under different storage mediums, along time.

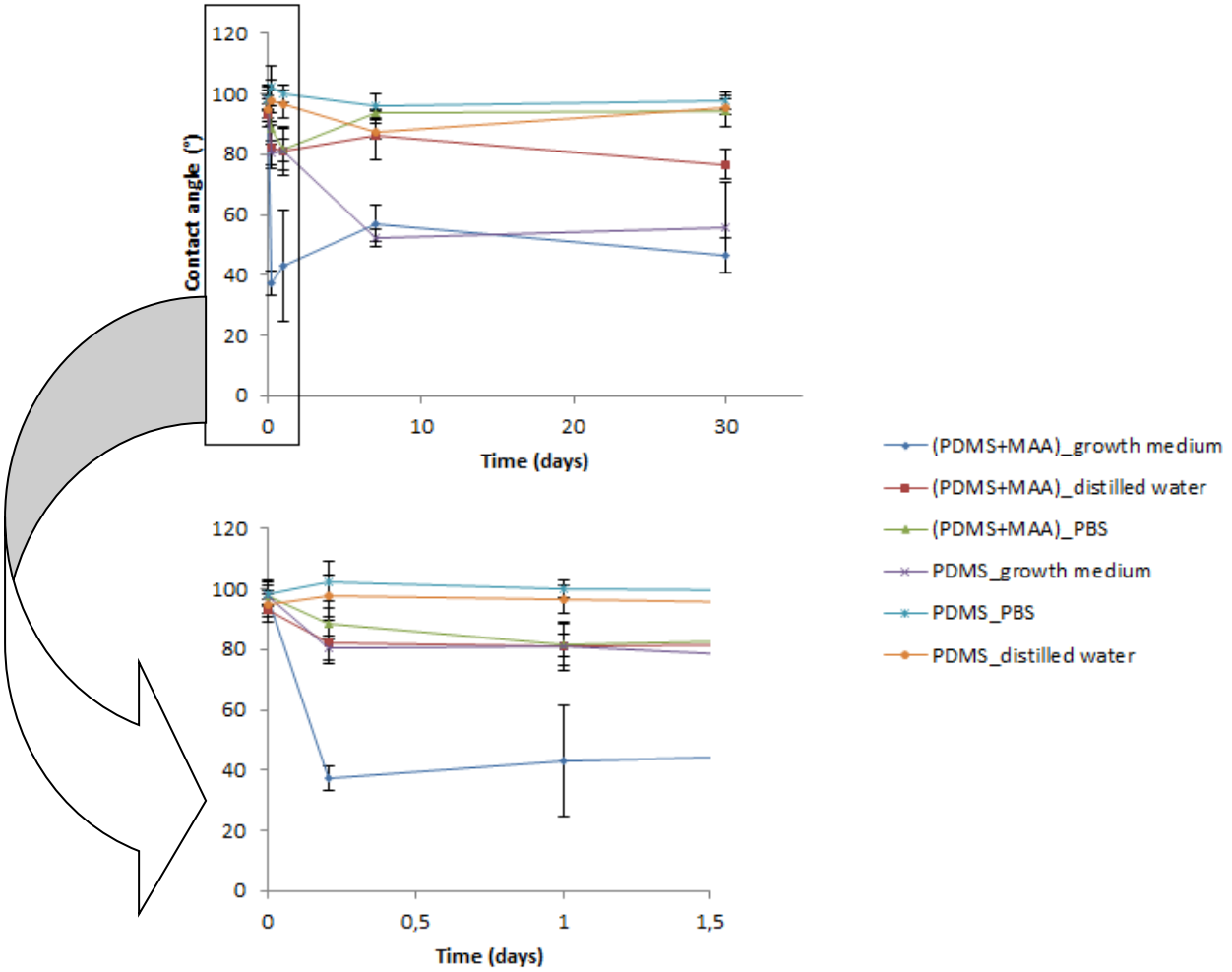

Figure 6.7 - Contact angle Plasma-induced graft copolymerization of MAA onto PDMS surface, under different storage mediums, along time.

6.2.4 Cytotoxicity tests

The human fibroblasts cells were seeded at the same initial density in the 96-well plates, with or without materials to assess its cytotoxicity. Cell adhesion and proliferation in the presence of the materials was characterized through an inverted light microscope (Figure 6.8).

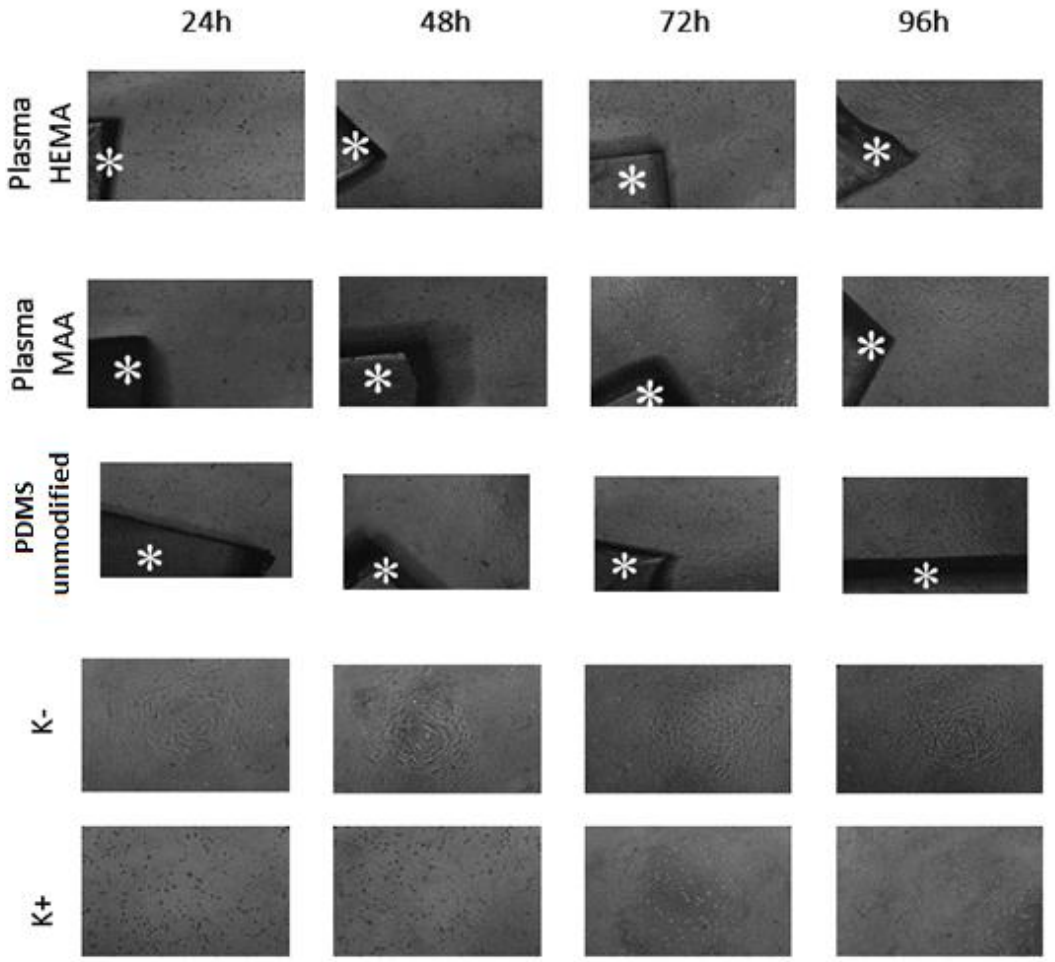

Figure 6.8 - Microscopic photographs of human fibroblasts cells seeded in the presence of the different PDMS materials (*) after 24, 48, 72 and 96h of incubation; K-, negative control; K+, positive control. Original magnification x100.

Figure 6.8 shows that cells adhered and proliferated in contact with all the materials and in the negative control. However, in the positive control no cell adhesion or proliferation was observed.

Furthermore, a MTS was also performed in order to further characterize materials cytotoxic profile. The MTS assay results (Figure 6.9) showed that cells in presence of tested samples had higher viability than in the positive control. After 4 days the cells remained viable, despite the end of this period the cells in contact with PDMS, suffered a decrease on its viability. Moreover, along time, the cell viability was always greater for the modified materials than for the unmodified PDMS.

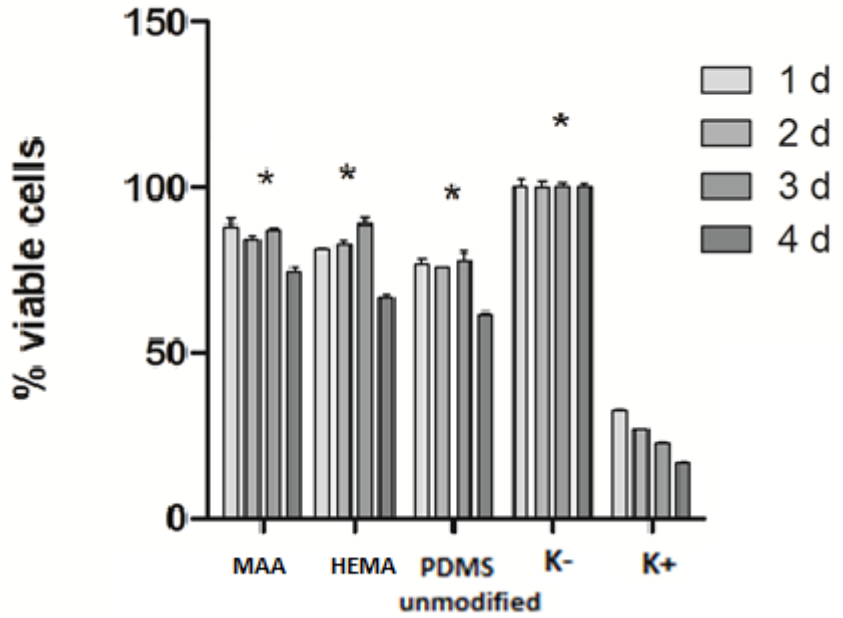

Figure 6.9 - Evaluation of the cellular activity of human fibroblasts cells seeded in the presence of the different PDMS materials after 24, 48, 72 and 96 h. MAA ; HEMA ; Positive control (K+); negative control (K-).Each result is the mean ± standard error of the mean of three independent experiments. Statistical analysis

6.2.5 Antibacterial activity tests

In order to observe bacteria presence on materials surface, SEM analysis was performed (Figure 6.10).

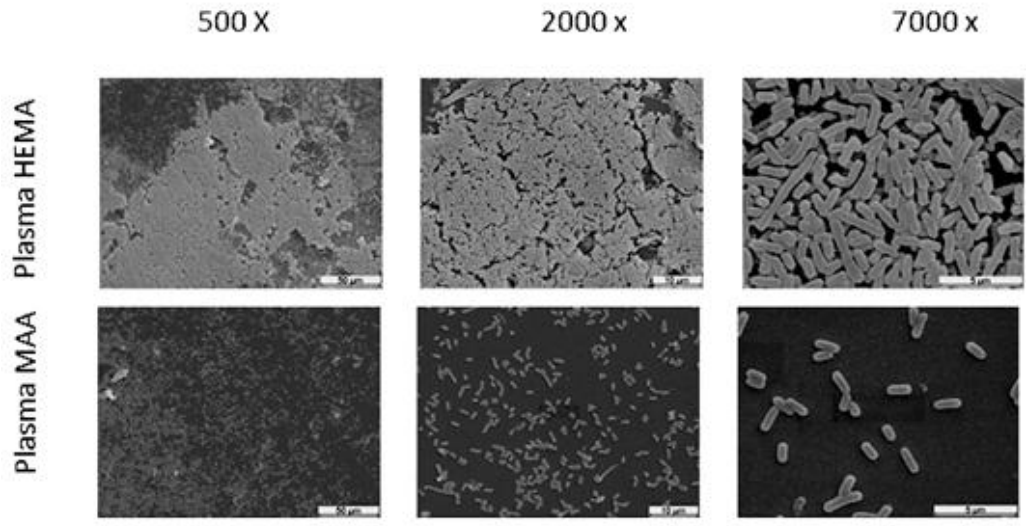

Figure 6.10 - SEM photographs of *E. Coli* seeded in the presence of the different PDMS materials at different magnifications 500 x, 2000 x and 7000 x.

Through this assay it is possible to observe the bacterial growth in all wells, which shows that these materials did not have an antibacterial effect. Nevertheless, The SEM analysis showed a reduction of the bacteria growth in PDMS+MAA material compared with PDMS+HEMA.

6.3 Discussion/ Conclusion

Plasma-induced graft polymerization of HEMA and MAA was found to be an effective technique in reducing the hydrophobic character of the surface, for both monomers. It happens because there was an increase of the polar component of the free surface energy, which means that this technique was successful on grafting polar groups on materials surface.

After 1 month, of materials being in contact with growth medium, PBS and distilled water, they presented a hydrophilic behavior similar to their original value. Nevertheless, the PDMS surface after modification, under the three storage conditions, proved to be more hydrophilic than before modification (PDMS unmodified).

Analogously to the UV modification, the percentage of swelling of PDMS grafted by MAA and HEMA was very low (less than 0,3%), shows means that the PDMS films have no tendency to swell in the presence of fluid, which can be explained by the cross-linked structure and the highly hydrophobicity that this material exhibits.

The samples of PDMS, after modification with HEMA and MAA, were found to be non-toxic, a condition necessary for biomedical applications. However, PDMS materials did not presented antibacterial activity which is fundamental to avoid biofilm formation at PDMS surface.

7. General conclusions

During this work, different techniques, such as UV grafting, aminolysis and plasma grafting were used for the modification of silicone based materials to be used as voice prosthesis.

PDMS (polydimethylsiloxane) surface was modified by UV (ultra-violet) grafting with hydrophilic monomers (Methacrylic acid and Hydroxyethylmethacrylate). The results obtained showed that there were no significant differences in the hydrophilic character of the surface (decreasing of the water contact angle approximately equal to 10°), especially when HEMA was used, as monomer.

The modification of PDMS surface with an amine (1.6-hexanediamine) reduced the water contact angle (decreasing of the contact angle approximately 50°), showing that the introduction of $–NH_2$ groups was successful. In FTIR spectra, the bands around 1600 cm^{-1} (for N-H bend) and 3350 cm^{-1} (for N-H stretch) were assigned to the introduction of $–NH_2$ groups to the PDMS surface after the modification with 1.6-hexanediamine. However, along time, under different storage mediums (distilled water, PBS and growth medium), PDMS surface recovered its hydrophobicity. A slight loss of weight was also observed on PDMS films. This could probably be assigned to a non efficient washing procedure of the films in this surface modification procedure, therefore, certain amine groups present on PDMS surface, that were not covalently attached could be present and were removed when the films were immersed in the storage mediums.

The best surface modification procedure studied was plasma grafting. With a previously study of the best conditions to activate the PDMS surface by plasma (regarding to working gas, processing time and pressure), HEMA and MAA were grafted on the surface. Regarding the hydrophobicity recovery, after one month, the hydrophilic character of the modified PDMS surfaces (stored under distilled water, PBS and growth medium) was maintained. Among all the evaluated storage mediums, growth medium showed to be the best option for the storage and stabilization of these modifications.

Moreover, all of the modified surfaces were found to be non-toxic, which is fundamental for biomedical applications. The results obtained for antibacterial activity characterization showed that none of the samples have antibacterial effects, was also possible to observe a reduction of the bacterial growth for both MAA grafted by plasma treatment and HEMA by grafted by UV. Unfortunately, the antibacterial activity tests are not complete yet. No results for unmodified PDMS films were available and therefore they were not taken into consideration to evaluate the effect of each surface modification technique in the bacterial adhesion. With this result, will be possible to see the difference of the bacterial growth on the PDMS surface, before and after each modification and a further analysis and conclusion can be made.

8. Future Work

Regarding the evaluation of the antibacterial activity of PDMS materials, samples grafted with MAA by plasma activation and films grafted with HEMA by UV, proved to be the most accurate on decreasing the bacterial growth. For the evaluation of the antibacterial activity, samples of the surface modified material were sent for analysis on normal petri dishes, in direct contact with air. However, in this research work, was showed that different storage mediums were able to maintain the hydrophilic character of the PDMS surfaces. For this reason, in the future, it would be advised to send the modified materials, under the best storage medium observed for each modification, in order to maintain all the surface properties of the material.

As future work, it would be interesting to study the grafting of other monomers, such as Ethylene glycol, acrylamide, vinylpyrrolidone. These monomers also have important properties such as biocompatibility and low toxicity. At the same time they are well known for preventing nonspecific adsorption of proteins. [99]

In order to further understand the information about the modified surfaces and their efficiency several other characterization techniques would be essential, such as: atomic force microscopy (AFM) to study the morphology of the surface and X-ray photoelectron spectroscopy (XPS) to evaluate the elemental composition of the surface.

The evaluation of the mechanical properties of the material before and after the modification would also essential in order to confirm if the bulk of the material was not affected by the surface modification procedure.

9. References

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