LASER INDUCED SURFACE MODIFICATIONS OF PDMS AS A BIO-COMPATIBLE MATERIAL

A thesis submitted in partial fulfilment for the degree of Doctor of Philosophy

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ABSTRACT

Surface modification of polydimethylsiloxane (PDMS) based vulcanizate rubber by CO_2-pulsed laser as the excitation source, without a photosensitizer, was studied at room temperature. The modified surfaces were characterized using a variety of techniques including scanning electron microscopy (SEM) combined with energy dispersive X-ray analysis (EDXA), attenuated total reflectance infrared (ATR-IR) spectroscopy and water drop contact angle analysis. EDXA showed that all of the treated PDMS surfaces contained a higher ratio of O/Si than the base PDMS. SEM micrographs and water drop contact angle variations showed the uniform porosity and high decrease in the wettability of the surface of PDMS respectively. The bulk mechanical properties of PDMS after laser-treatment did not change, as shown by dynamic mechanical thermal analysis (DMTA).The friction coefficient of the surface of the modified silicone decreased drastically, even after only one pulse was delivered to it.

Data from *in vitro* blood compatibility experiments indicated a significant reduction of platelet adhesion and aggregation for the modified surfaces and those platelets which were adherent remained unspread (no activation). The extent of reduction of platelet adhesion was correlated to the number of laser pulses.

The attachment of anchorage dependent cells, namely Baby Hamster Kidney (BHK) fibroblastic cells was investigated under stationary culture conditions. The laser treated surfaces showed little adhesion, no spreading and growth properties. This technique can be employed to prepare PDMS samples in which one surface is laser treated and the other is untreated. Such materials may be useful in, for example, medical implants in which one (the treated) surface is in contact with a blood supply and hence does not cause cell aggregation (clotting) and the other side is tissue compatible (allows adhesion

of tissue).

Acrylamide (AAm), 2-hydroxyethylmethacrylate (HEMA) and hydroxyethylmethacrylate phosphatidyl choline (HEMAPC) were grafted onto pre-irradiated PDMS. Platelet adhesion and cell attachment studies show that the biocompatibility of the AAm and HEMA grafted PDMS are intermediate between that of untreated PDMS and either the HEMAPC grafted PDMS surface, or PDMS surfaces that had been treated with 10 CO_2 laser pulses.

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CHAPTER 1

1 INTRODUCTION

The properties of a polymeric surface are important in many applications, including chemical resistivity, adhesion, wettability, permeability and biocompatibility. As a consequence, it would be helpful to be able to treat the surface of a polymer, which has optimum bulk properties, in order to be able to make it suitable for the desired application. In recent years, a large number of investigations have been undertaken to develop biomedical prostheses which do not elicit thrombus formation when exposed to flowing blood. However, to date, no thromboresistant material that is reliable for long term use has been reported, largely because many parameters govern platelet aggregation and blood coagulation. Among them are the surface properties of the material and the hydrodynamic properties of flowing blood[1].

Extensive works on blood compatibility of polymeric materials have revealed that it is strongly governed by their surface structure and properties[1,2]. Among them are roughness, hydrophilic-hydrophobic balance, ionic concentrations and species[1].

Interactions between the coagulation system and the polymer surface are of a highly complex nature and depend on the relative blood compatibility of a biomaterial[3]. These interactions involve plasma enzymes as well as cellular elements and flow conditions. Since platelet adhesion to a biomaterial surface is important, as it results in the formation of haemostatic plug or thrombus, counting platelet numbers attaching to a surface is one of the most popular experimental methods for evaluating the

`hemocompatible' properties of man-made materials[4]. Several strategies have been proposed to improve the blood compatibility of biomaterials. One strategy involves the synthesis of a highly hydrophilic interface by grafting hydrogel groups to the backbone of a hydrophobic polymer.

Another approach to thromboresistance includes the introduction of highly hydrophobic groups to the blood contacting interface by grafting alkyl chains to relatively hydrophilic materials. There is a general agreement that *in vitro* tests are most useful in the evaluation of artificial surfaces that are highly reactive with blood. It is also agreed that human blood should be used for *in vitro* tests whenever possible. The evaluation of hemocompatibility *in vitro* usually involves platelet assays and coagulation assays[5].

In this thesis we undertook a study to examine the reduction of platelet adhesion on a polymer surface after prior treatment, using a laser irradiation method to alter the surface properties of the material. Interactions of these blood components with unmodified man-made materials will trigger thrombus formation on the foreign surface when it comes into contact with blood. The rationale for surface modification of PDMS by laser treatment, to reduce platelet adhesion, is based on the observation of `super-hydrophobic' surface properties of samples of polydimethylsiloxane (PDMS) polymers afeter certain laser-irradiation treatment. A major advantage of using the laser-induced surface modification of polymers provides lies in altering the surface *without* altering their bulk properties[6,7]. Surface modification by the use of laser treatment also has the advantage of ease of treatment of certain surfaces that are difficult to treat by

conventional chemical methods. The resulting surfaces are particle-free and sterile, due to the low amount of chemical introduced[6,7,8]. The deposition films are thin, tightly adherent and can be deposited on complex geometries. This technique offers possibilities to improve the performance of existing biomaterials and medical devices and for developing new biomaterials[6,7]. Other advantages of laser sources to initiate chemical reactions have previously been reported[6,7,8,9].

Irradiating a polymeric material with a continuous laser beam can overheat the surface and may damage both the surface and the bulk properties of the polymer. In contrast, pulsed lasers allow short exposure times and, by optimizing the time intervals between pulses (repetition rate), the laser fluency and pulse number[6,7,8,10], less the risk of thermal damage.

Rubbers based on PDMS have some excellent properties such as high structural resistance towards heat, ozone and chemicals[11]. Medical-grade silicone rubber is a widely used biomaterial for different applications including tubing, catheters, vascular grafts, plastic reconstruction, encapsulation of electronic components and voice prostheses[12,13]. For long-term applications of the silicones used, the stability and the biocompatibility of the polymers are crucial[12].

It has been reported that, for biocompatibility, the polymer surface, should either be 'super-hydrophilic' or 'super-hydrophobic', indicated by very high or very low surface free energy. It is well known that thrombus formation is triggered by an interaction between blood components and the foreign polymer surface[14]. On the other hand, protein adsorption depends greatly upon the surface energy of the substrate[15]. In the

present work we have utilized a line-tunable pulsed CO_2-laser to induce the surface modification of PDMS to create a (super-hydrophobic) polymer surface, so reducing its surface free energy of PDMS to improve blood compatibility, while keeping the bulk properties of the substrate intact. The surfaces of the treated samples have been characterized and their surface morphology studied. To our knowledge, no work on the surface modification of PDMS with a laser in order to improve blood compatibility has previously been reported.

CHAPTER 2

LITERATURE SURVEY

2.1 SURFACE MODIFICATION

2.1.1 INTRODUCTION

For longer than half a century, polymeric materials used in industry have been subjected to various surface modifications. Table 1 lists the general methods used for surface modification of polymers, some of which are no longer used because of high cost or environmental protection[1].

Physical modifications of polymer surfaces can be divided into two main categories, the first involved with chemically altering the surface layer, the second with depositing an extraneous layer on top of the existing material, thereby generating a new interface. Given the non-reactive character of polymer surfaces, the former requires means for generating high energy species, *e.g.* radicals, ions, molecules in excited electron states, *etc.*

Much attention has recently been paid to the modification of polymer surfaces. The surface material is sometimes more important than the bulk phase of the polymer. Solids communicate with other materials only through their surfaces. Many properties

of a polymer, *e.g.* adhesion, printability, dyeability, wettability, chemical resistance, antistatic behaviour, permeability, biocompatibility and blood compatibility are not related to the bulk properties of the material but to its surface layer[16]. Therefore, surface modification of a polymer possessing optimum bulk properties offers an efficient way to render it suitable for special purposes. However, radiation induced chemistry is an efficient method for polymer surface modification and over a hundred articles have been published in this field, which are presented in this review.

Table 1 General methods for polymer surface modification.

Examples	
Roughening	Sand blast, Etching
Oxidation	Alkaline treatment, Chromium treatment, Fire exposure, irradiation technique
Coating	Casting, Lamination, Plasma polymerization
Blending	Surfactant addition, block and graft copolymer addition
Ion implantation	High energy argon and nitrogen injection
Graft polymerization	UV, Ionizing radiation, Low temperature plasma

2.1.2 MODIFICATION TECHNIQUES

2.1.2.1 Corona treatment

Corona treatments exploit the corona effect, *i.e.* the formation of high energy electromagnetic field close to charged thin wires or points, with consequent ionization in their proximity, even at atmospheric pressure and relatively low temperature[17,18]. In the ionized region excited species (ions, radicals, electrons, molecules in excited states, *etc*) are present and the latter are active in surface modification, typically introduction of oxygen containing functions. Flat films or sheets or wires can be conveniently used. Most polyolefin films for packaging purposes are routinely corona treated[19]. The disadvantage with corona treatment is the limited control possible on the process[20].

2.1.2.2 Plasma Treatment

Plasma treatment is an important step used in microelectronic fabrication and in biomaterials technologies and a wide body of literature is involved with plasma etching of organic and inorganics materials. An important area is in biomaterials, where the possibility of unusual chemistries when plasma polymerizing allows lots of combinations in terms of coating chemistry; another very important issue is ageing[21-25]. It is known that after treatment the induced surface changes are partially reversible with time (hydrophobic recovery) and this is particularly important from an application point of view. Typically using polymer surfaces as a substrate for plasma

treatment has both an advantage and a disadvantage. The former lies in the fact that chemical bonds at the interface can be formed. The latter hinges on the possible high crosslinking of the plasma polymerized layer, which can make them mechanically incompatible with the substrate[26].

Plasma treatment and plasma deposition polymerization provide a method for the surface chemical modification of polymeric materials [27,28]. These techniques offer the possibility of improving existing biomaterials[29]. Modifying the polymer surface by graft copolymerization has also been made possible by using the free radicals or peroxides generated on the surface by plasma treatment[30].

2.1.2.3 UV Treatments

Most applications involve photon-activated cross-linking (negative resists, paper coatings) or fragmentation (positive resists) of the polymer coating. Sensitizers are typically added to enhance the photon yield of the processes. Most commodity polymers have been extensively studied in terms of their photo-oxidative behaviour. Examples are PP[31], PET[32] and PC[33]. UV light initiated grafting has been studied by Yao *et al* [16] Hyakaw *et.al* [34]. have reported graft copolymerization of maleimide onto ethylcellulose and polyethylene film by using its sublimation vapour under UV irradiation.

Extensive UV grafting using the simultaneous irradiation method has been reported, however, using naturally occurring macromolecules such as cellulose[35,36] and wool[37] and some synthetic polymers including polyolefins[38-42].

In practice, the most efficient UV grafting processes are performed by adding a specific

photosensitizer to the monomer/ polymer system[41-43].

2.1.2.4 X-ray and Gamma Ray Treatments

These cannot really be considered surface treatments, since, as the photon energy is very high, the photon mean free path is also quite high. Yet applications similar to UV treatments have been reported, involving mainly cross-linking of polymeric coatings.

Furthermore, in the presence of oxygen, high energy photon treatment (*e.g.* for sterilization purposes) induces the formation of radical sites at surfaces, which then react with atmospheric oxygen forming oxygenated functions[44].

The weak point in using high energy radiation such as the gamma or electron beam as excitation source for grafting copolymerization is that the bulk mechanical properties of the substrate may change[6,45]. The styrene butadiene styrene grafted to hydroxyethylmethacrylate (SBS-g-HEMA) synthesized by Co-gamma ray irradiation has been described and has been characterized by infrared spectroscopy and scanning electron microscopy[46].

2.1.2.5 Electron Beam Treatments

A few patents and papers suggest the use of low energy electrons for surface treatment, aimed at improving adhesion by mechanically strengthening the surface layer and introducing some oxygen at the surface by the formation of radicals and consequent reaction with atmospheric oxygen[47-49].

2.1.2.6 Ion Beam Treatment

Ion beams can be used for two different purposes, namely to directly alter the surface composition of the material or to sputter off target species which are then deposited on the surface. Patents are few and are subordinate to ion implantation on plastics to improve wear resistance or biocompatibility[50,51].

2.1.2.7 LASER TREATMENT

2.1.2.7.1 Introduction

Lasers are photon sources characterized by energy and space coherence, with intensities which can be dramatically high (in the MW range). Similar in effect to conventional photon sources, they can be used to promote cross-linking or scission effects. Recently some applications to adhesion improvements have been reported, involving the use of IR lasers for surface cleaning. A number of papers have been devoted mainly to laser ablation[52-55]. The aim of published papers is in general to discriminate between photochemical and thermal effects in CO_2 laser and excimer laser-induced ablation, whilst measuring the dependence of the etch rate upon laser parameters (*e.g.* frequency, fluence)[56-58].

2.1.2.7.2 UV LASERS

Technologically, UV laser treatment can lead to interesting modifications of

surface properties, such as better wettability, adherence and printability of polymers. As reported, PE, PP, PVF, PS were treated in air with an argon-fluorine UV excimer laser[59].

From the fundamental point of view, UV laser treatment of a polymer can involve two different mechanisms, either a photothermal energy conversion or a direct photochemical process. The relative importance of these processes varies with the wavelength of the laser source[60,61] and the nature of the polymer. The photochemical process consists of direct chemical bond breaking by one or several photons[59].

Photochemical processes of polymers commonly involve UV laser treatment. The photothermal process is the most common interaction process of laser with the matter. In this case, the light is used as a powerful and versatile source for heat treatment; for example, for laser annealing in the recrystallization of polycrystalline silicone in the microelectronics industry[59,62].

Published experimental work gives evidence that polymers exposed to a UV laser beam can react in two different ways, either, through a direct photochemical or through a photothermal conversion of photon energy, or both; whether one or the other mechanism-dominates depends on the absorption coefficient of the polymer at the wavelength used and on the beam fluence (energy per unit area)[59,63].

The laser-initiated polymerization of vinyl monomers using different types of laser has been reported in earlier publications[64-68]. There are several advantages in utilizing lasers to initiate polymerization, including the ability to tune the laser to a specific wavelength, thus exciting a particular chemical bond without dispersing energy

throughout the reaction[7].

2.1.2.7.3 CO_2 LASERS

The use of a CO_2 laser in polymer etching provides certain advantages over the excimer laser: large pulse, large beam sizes, higher laser efficiency, and the use of nontoxic gases. The chief limitation is that the limited range of IR emission wavelengths prohibit its use for exciting chemical bonds[69].

Etching of thin polyimide films in air have been investigated using a line tunable pulsed CO_2 laser. The threshold fluence for etching at a wavelength of 944 cm^{-1} (10.6 μm) is greater than that required at 1087 cm^{-1} (9.2 μm) by a factor of 4. This is consistent with the infrared absorption spectrum which shows polyimide to be significantly more absorbing[69] at 1087 cm^{-1}.

CO_2 laser photopolymerization in the liquid phase in the presence of the radiation absorbing photoinitiators is a well known phenomenon[70].

The explosive decomposition of the (fluoromethyl)silanes H_2CFSiH_3, HCF_2SiH_3, CF_3, SiH_3, and $(CF_3)_2SiH_2$, which furnishes Si/ C/ F/ H containing deposits, has been induced by irradiation with a single CO_2 laser pulse at pressures of 0.1-6.7 KPa[71].

Infrared multiphoton decomposition of the fluoromethyl-silane can be induced at pressures of 0.1-6.7 KPa with appropriate irradiating lines tuned to absorption bands at fluences 0.2-0.9 J/cm^2. The irradiation with a single pulse results in an explosive reaction when the pressure exceeds a certain limit, which depends on the laser fluence and the wavelength[71].

The gas phase thermal decomposition of trimethyl(methoxy) silane, hexamethyldisiloxane and tetra-methoxysilane, induced by a continuous wave CO_2 laser and photosensitized by SF_6, leads to a variety of gaseous products and solid deposition that incorporate most of the silicone from the parent compounds[72].

The technique of the CO_2 laser induced decomposition of silane in the presence of oxygenated olefins can find application as a method for the preparation of new organosilicone powders and adds to the potential of the infrared multiphoton dissociation of silane to form new silicone containing materials[73].

As reported[74] chemical reaction induced by CO_2 laser radiation of mixtures of silane and hexafluoroacetone afford various gaseous silicone and carbon containing compounds and result in deposition of microstructures of carbon, C/ F/ O and Si/ C/ O/ F materials[74].

An effort has been made to use some CO_2 laser induced chemical reactions of silane and decompositions of strained organosilicone compounds for the production of organosilicone polymer coatings[75-78]. Thermal decomposition of trimethyl(methoxy)silane (TMMS), hexamethyldisiloxane and tetramethoxysilane induced by continuous wave CO_2 laser radiation in the presence of energy conveying sulphur hexafluoride has been reported[72].

The laser-induced chemical vapour deposition of organosilicone polymers is one aspect of current research on laser driven production of novel silicone containing materials. Two types of these high molecular weight compounds have been prepared in this way so far, namely (a) organosiloxanes obtained by infrared laser multiphoton

decomposition of silane in the presence of some common monomers, and (b) polycarbosilanes, formed by infrared laser photosensitized decomposition of silacyclobutanes and methoxysilanes[79].

The usefulness of CO_2-pulsed lasers for polymerization and grafting of vinyl monomers such as acrylamide onto polymer substrates has been reported[7].

2.2 MULTIPHOTON DISSOCIATION

2.2.1 Introduction

Stimulated resonant transitions in matter under the action of laser light have very important properties for applications in chemistry. First, the rate of excitation due to stimulated transitions m ——▸ n is proportional to the light intensity [80]

$$W^{nm}_{exc} = \sigma_{nm} I(\omega_{mn})$$

where σ_{nm}, ω_{mn} are the cross-section and frequency of the resonant transition between the quantum levels "m" and "n". This allows a very high rate of energy deposition into an atom or a molecule (1-10 eV during 10^{-8} - 10^{-12} (s), i.e. up to 10 ^{13} eV/s) that greatly exceeds the relaxation rate W_{relax}.

On condition that $W_{exc} >> W_{relax}$ it is possible to excite considerably the internal degrees of freedom (vibrational and / or electronic) of atom or molecule without heat. Secondly, the resonant character of stimulated transitions enables exciting atoms or molecules of a definite sort in mixtures, that is, providing intermolecular excitation selectivity. Thus,

laser resonant excitation prepares a substance in a highly non-equilibrium state with relation to both different degrees of freedom and particles of different sorts in a mixture[80].

It is now generally understood that although a polyatomic molecule has discrete energy states at low temperatures, the density of states increases very rapidly with increasing temperature and the states soon form a quasi-continuum[81]. It is believed that a moderately strong laser field can selectivity excite the molecule over the discrete states via a resonant multiphoton transition and even through the quasi-continuum via resonant stepwise transitions to and beyond the dissociation threshold. The initial excitation over the discrete states depends not only on the frequency and the power of the laser, but also on the rotational quantum states and the excitation of hot bonds and is mainly responsible for the high selectivity of the process. Once the molecules are excited to the quasi-continuum, the energy fluence, not the power of the laser, was shown to be responsible for driving the molecules through the quasi-continuum and determining the yield of dissociation. For molecules lying above the dissociation level, the rate of decomposition competes with the rate of excitation and then the power of the laser should determine the average level of excitation (or energy deposition). Since the invention of the laser in the early 1960s, along with the arrival of various laser accessories and optical devices, there have been significant advances in the understanding of the interaction of photons with matter. But, probably none of the phenomena discovered so far in the general area of laser chemistry and quantum electronics is as exciting as the infrared multiphoton excitation and dissociation (MPE

and MPD) of polyatomic molecules[81].

The motivating idea behind laser chemistry is its potential for modification of chemical properties of molecules through the absorption of laser photons. UV and visible photons are most effective in fulfilling this purpose by causing electronic transitions in molecules and there has been much interesting work in recent years, especially using tunable dye lasers. The absorption of an infrared photon by a molecule is not likely to induce as drastic a change in the chemical properties as UV or visible photons but, on the other hand, it offers an exciting possibility of promoting one specific reaction channel over others by exciting a particular mode of molecular vibration that is directly or closely related to the motion along the reaction coordinate.

Multiphoton excitation (MPE) could be a novel method for energizing molecules, one that offered the potential for vibrational mode control of molecular decomposition. In other words, by depositing energy into particular vibrational modes, one hopes to dissociate molecules along certain reaction pathways different from those of thermal decomposition[81,82].

2.2.2 Radical Production by MPD

The efficient and inexpensive production of molecular radicals by MPD may stimulate further understanding of radical reactions. Laser control of the identity of and excitation in molecular radicals may be used to study radical reactions and provide some practical new synthetic methods. Multiphoton excitation of polyatomic molecules can complement conventional high temperature chemistry of polyatomic molecules.

The first reported primary product analysis for the multiphoton dissociation of SF_6 indicate that this molecule dissociated into SF_4 and F_2 [81,82].

Recent progress has occurred in the synthesis of C_2H_3Cl (vinyl chloride) by excimer laser-induced radical-chain reactions. This process is of considerable industrial importance, being the main route in the production of vinyl chloride monomer feedstock for PVC manufacture. In this case the excimer KrF laser generates the enhanced concentration of free radicals which control the chain reaction of 1,2-dichloroethane (DCE) to vinyl chloride (VC) conversion. The experiments demonstrate clearly the effect of laser-generated free radicals in the DCE to VC conversions used in the technical process[80].

The heating of a molecule via MPE is much more rapid (on a nanosecond time scale) than under any other experimental conditions. Moreover, selected components of a reactant mixture can be singled out and heated without wall effects, primarily internal vibrational energy and little rotational and translational energy is given to the molecule. These features are peculiar to IR laser induced chemistry[82]. Differences in vibrational and translational temperatures is individually discussed[80].

2.2.3 Types of Photoexcitation

The methods of laser-induced chemistry can be classified according to the type of photoexcitation. All methods of single-photon linear photochemistry are based on the excitation of the electronic state of the atom or molecules (single-photon electronic photochemistry) or the vibrational state of the molecule (single-photon vibrational

photochemistry) as one photon is absorbed. The excitation of atomic and molecular states by visible UV irradiation of ordinary sources is well known in photochemistry[80]. Laser radiation is very useful for two reasons. First, the high spectral brightness of laser light makes it possible to excite any discrete quantum state of an atom or molecule without accidental coincidences between the wavelengths of the intense lines of spontaneous radiation of ordinary sources and the absorption lines. Second, the high spectral brightness of IR lasers permits the excitation of molecular vibrations.

The high intensity of laser radiation has allowed new methods of multiphoton (MP), nonlinear photochemistry to be developed, which have no analogy in conventional photochemistry. These methods are based on absorption of a number of laser photons by one particle (an atom or a molecule). Techniques of multistep excitation [80,81] of atomic and molecular electronic states have needed to be developed. These methods were based on the ability of laser light to transfer a considerable fraction of atoms and molecules from the ground state to the excited state when the absorption of photons by excited particles becomes probable.

2.2.3.1 UV/visible laser - Induced Curing of Acrylic Coating

Lasers will be primarily used as a high intensity UV source to produce radicals by a photochemical process, much like in conventional UV curing. Laser-induced polymerization of monomers has attracted significant attention in recent years and numerous papers have been published dealing with the capability of both pulsed and continuous lasers to initiate polymerization[8,83,84]. Photosensitized polymerization of

acrylates and acrylamides has been produced by argon, helium-neon and ruby lasers[10,85,86].

The capability of UV lasers to induce the polymerization of multiacrylate monomers was demonstrated for both continuous and pulsed irradiation by Decker[68]. A nitrogen-pulsed laser has been successfully used for ultrafast polymerization of epoxy-acrylate photo resists[87]. Williamson, Smith and Castle have investigated 24 combinations of donor/acceptor systems for copolymerization using nitrogen and argon lasers[88]. Laser-initiated copolymerization of N-vinyl pyrrolidone (NVP) with maleic anhydride and maleimide has been carried out using a CW argon ion laser[67] in the presence of nitrogen at different laser irradiation times. The laser was operated in the UV mode (363.8 nm) and the samples were exposed at a power density of 1.2 W/cm^2. The critical role of laser pulsing frequency in the molecular weight distribution of methylmethacrylate generated with a high-intensity pulsed laser has been measured[89]. In a U.S. Patent, frequency-doubled, ruby laser light has been used in the sensitized polymerization of vinyl acetate[90]. The laser-initiated polymerization of vinyl monomers and epoxies using a CW argon ion laser has also been reported[9].

2.2.3.2 IR laser-Induced Chemical Radical Synthesis

The discovery of the phenomenon that intense infrared radiation of correct frequency can selectivity and efficiently deposit tens of photons in isolated molecules and cause dissociation is an exciting breakthrough. The possibility that a polyatomic molecule placed in the intense field of an infrared laser can absorb enough photons to

dissociate in a very short time span was first suggested, in 1971, by Isenor and Richardson based on their experimental observation of luminescence from dissociation products[81].

IRMP processes can be observed in polyatomic molecules of any complexity and symmetry if they have more than four or five atoms and have IR absorption bands in the spectral region of lasing of high-power IR lasers. Most experiments on MP excitation and MP dissociation have been carried out with TEA CO_2 lasers (λ=9-11 μm). The universality of IRMP processes allows the dissociation of many various molecules and the production of a wide variety of free radicals and provides a potential basis for a variety of processes for IR laser radical chemical synthesis.

Intense IR radiation is able to deposit the energy essential for the dissociation of wanted molecules in a mixture selectively, without depositing such high energy into the other molecules in the mixture. This is caused first, by the strong difference in MP absorption spectra in different molecules and, second, by the possibility of realizing collisionless excitation during an IR pulse when there is no significant collisional energy transfer from the excited molecule to the other molecules in the mixture[80].

The development of high-intensity IR lasers made it possible to realize collisionless, multiple-photon excitation of high-lying vibrational levels of polyatomic molecules by intense IR radiation (10^6 - 10^8 W/cm^2) [80,81]. Infrared-laser induced reactions with a pulsed CO_2 laser have also been extensively studied for many molecules that absorb the infrared light in the 9-11 μm region. Reactions of molecules that do not appreciably absorb the CO_2 light have been made possible by adding an infrared photoinitiator

and/or sensitizer which strongly absorbs the laser light without participating in the chemical reactions. Several workers showed that some sensitizers such as SF_6 were decomposed by the CO_2 laser quite easily and that these molecules could be used as the sensitizers only when the energy of the CO_2 laser beam was low. Koga et.al found that a considerable amount of SF_6 was decomposed by the CO_2 laser with fluence as low as 0.7 J/cm^2 and consequently, they recommended that the fluence should be below 0.3 J/cm^2 when this molecule was used as the sensitizer[91].

The intense IR radiation of a pulsed laser at a frequency tuned to the vibrational absorption bond of any polyatomic molecule induces very fast vibrational excitation of that molecule[80,81,91]. In other words, under a resonant IR field the vibrational degrees of freedom are subjected to strong heating which depends on the number of IR photons absorbed by the molecule. Under the action of an IR pulse with its energy fluence varying from 1 to 10 J/cm^2 it is relatively easy to deposit energy of 1-10 eV into a polyatomic molecule via the absorption of, say, 10-100 photons from a CO_2 laser operating in the region of 10 μm (1000 cm^{-1}). For such a strong vibrational excitation the rate of photoexcitation W_{exc} and the laser pulse duration, τ must satisfy the following requirements[80]:

$$W_{exc} = \sigma_{abs} I > \tau_p^{-1} > W_{relax}$$

Where σ_{abs} is the average cross-section of successive vibrational upward transitions of the molecule, W_{relax} is the relaxation rate of vibrational states. This condition of efficient

excitation is valid when the laser pulse energy fluence $\emptyset = I\tau_p > \sigma^{-1}_{abs}$ (photon/cm^2). For effective absorption of n photons by the single molecule the energy fluence Ø of laser pulse evidently should satisfy the more stringent condition: $\emptyset > n/\sigma_{abs}$ (photons/cm^2). The IR MP absorption cross-sections for different polyatomic molecules lie in the range $\sigma_{abs} = 10^{-18} - 10^{-20}$ cm^2.

The first experiments on IR laser radical chemical synthesis were performed many years ago. The isotopic selectivity of IRMP molecular dissociation was first demonstrated when the B_2O_3 molecule was synthesized by the reaction of oxygen with the IR MP dissociation products of the BCl_3 molecule. Later, Clark *et al.* demonstrated[80], the possibility of radical-radical synthesis of the SF_5NF_2 molecule by acting on a mixture of S_2F_{10} and N_2F_4 molecules with a powerful IR laser pulse[81]. This pulse, with a duration of $\tau_p = 10^{-7}$s, dissociated both of the initial molecules. The reaction scheme was:

$$S_2F_{10} \xrightarrow{n h\upsilon\ IR} 2SF_5^{\bullet}$$

$$N_2F_4 \xrightarrow{m h\upsilon\ IR} 2NF_2^{\bullet}$$

$$SF_5^{\bullet} + NF_2^{\bullet} + M \longrightarrow SF_5 - NF_2 + M$$

where M is the third body (a molecule, or a cell wall).

The infrared multiphoton decomposition of diethyl ether (DEE) has been investigated[92].

The CO_2 laser induced reaction of molecules such as SF_6 with silicone via vibrational

excitation and multiphoton absorption has been reported[93]. The wavelength dependency of laser induced interaction of molecules such as CDF_3 with the surface of SiO_2 has been widely investigated by others[94]. CO_2-pulsed laser-induced reactions of molecules that weakly absorb within the infrared region has been made possible by the addition of a sensitizer such as C_6F_6 and THF which strongly absorbs the CO_2 laser light via a multiphoton mechanism. Etching of thin polyimide films in air was investigated using a line tunable, pulsed CO_2 laser at a wavelengths of 9.2 μm (1086 cm^{-1}). This is consistent with the infrared absorption spectrum which shows polyimide has strong absorption at 9.2 μm[95].

2.2.3.3 Infrared Multiphoton Dissociation (IRMPD) Mechanism

Infrared radiation can be used efficiently when the wavelength of the beam corresponds with the vibrational energy of a specific bond of a molecule which participates in the polymerization processes and then a resonant situation is achieved in the reactions which leads to the cleavage of selected bonds. The most promising method in selective type of chemical reactions in solution systems is the resonance interaction between infrared laser radiation and the vibrational mode of the structural groups whose dissociation results in photo-polymerization. In other words, the deposition of intense infrared radiation of the correct frequency can selectivity and efficiently deposit tens of photons in isolated molecules and cause dissociation[81].

The qualitative model of the MP vibrational excitation and dissociation of an isolated molecule under the action of an intense IR radiation pulse was developed around a

decade ago[80,82]. According to the model, the molecule in the course of its vibrational excitation passes through the following three qualitatively different vibrational energy regions:

Figure 2.1 shows a multiphoton dissociation process involving closely spaced intermediate states forming a " quasi-continuum".

1) The region of low-lying discrete vibrational-rotational levels wherein a single transition is possible from a given state,

2) The vibrational quasi-continuum (QC) region wherein many close vibrational levels interact and many transitions are possible from a given state.

3) The real continuum region above the dissociation limit wherein the unimolecular decay of the vibrationally overexcited, isolated molecule becomes possible.

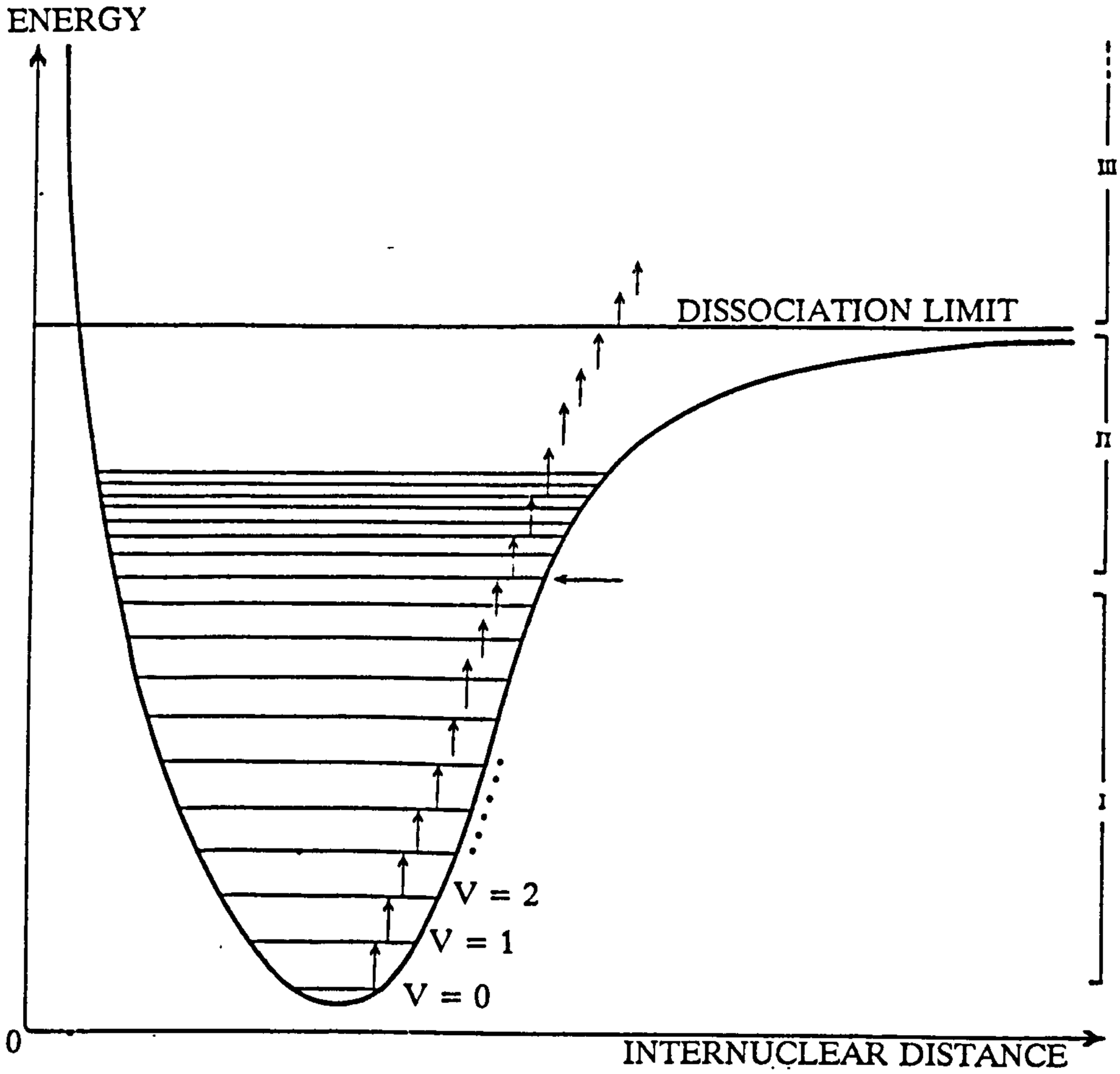


Figure 2.1 **Energy levels and transitions involved in the multiphoton infrared dissociation of a diatomic molecule. Note that each vibrational level has rotational fine structure (not shown) and also the energies are not discrete but have finite linewidth; both these features are important in the excitation process.**

2.3 Shortcomings of Ionizing Radiation

The weak point in using high energy radiation such as gamma or electron beam

as excitation source for grafting copolymerization or/ and surface modification is that the bulk mechanical properties of the substrate may change[96].

Physical and chemical changes in polymers and mechanisms for cross-linking and chain scission under exposure to radiation energies were established an of first described in radiation-treated polyethylene by Charlsby in 1954[97,98]. Also changes in the mechanical properties of polymers irradiated in air have been reported[99,100]. The tensile strength and the elongation of the sample irradiated in vacuum decreased also with storage time[101]. Polymers with ethylene sequences such as polyethylene (PE) and ethylene-propylene copolymer (EPR) are able to crosslink by high energy radiation and giving changes in its mechanical behaviour[102]. Since polypropylene (PP) is a semicrystalline material, a high population of radicals remain in the crystalline regions which slowly migrate to the crystal surfaces where oxidation takes place causing even further change in the bulk properties of the material[103].

Some materials, like PE, predominantly showed in crosslinking whilst others such as polyisobutylene were rapidly degraded by main chain scission[104]. The mechanisms for these are well established by Charlesby[105,106]. However, this potential shortcoming can be avoided by using the CO_2-pulsed laser source[45].

Infrared radiation can be used efficiently when the wavelength of this radiation matches the wavelength of vibrations of reactive groups, when a resonant situation is achieved in the reactions which involve breaking of bonds. The most promising method of achieving selected chemical reactions in condensed media is the resonant interaction between infrared laser radiation and the vibration of these groups whose dissociation

results in chemical reactions. For example, an investigation was made of the influence of laser radiation on the degree of completion of the imidization of a polyamide acid, prepared from the dianhydride of pyromelitic acid and 4,4'-diaminodiphenylester in dimethylformamide[107].

2.4 SILICONE RUBBER

2.4.1 Properties and Structure

The properties of silicone rubber are due to the unusual molecular structure of the polymer, which consists of a backbone of silicon atoms alternating with oxygen atoms. The silicon-oxygen-silicon linkage in silicones is similar to the linkage in quartz and glass. The mobility of the methyl groups as well as the larger volume of a silicon atom relative to a carbon atom results in a large free space for the dimethylsiloxane unit and limits the close approach of neighbouring molecules. Silicone rubbers have low glass-transition temperatures and high permeability and high compressibility. The stable bonding means that relatively long service life can be expected at elevated temperatures, as shown in Table 2.2.

The ozone and corona resistances of silicone rubber are outstanding, approaching that of mica[11,108].

Samples of PDMS have been exposed to outdoor weathering for 15 years with no significant loss of physical properties. This demonstrates its unique resistance to

Table 2.2 Estimated service life of silicone[11].

90 °C (194 °F)	40 years
121 °C (250 °F)	10-20 years
150 °C (300 °F)	5-10 years
200 °C (392 °F)	2-5 years
250 °C (482 °F)	3 months
315 °C (600 °F)	2 weeks

temperature extremes sunlight, water, and ozone and other gases. The rubber also has good resistance to low concentrations of acids, bases, and salts. Silicone rubber is odourless, tasteless and nontoxic when properly fabricated. Furthermore it does not stain, corrode, or in any way deteriorate materials with which it comes into contact[11]. Silicone based water repellents were introduced on the market in the 1950's. The advancing water contact angle on PDMS (about 112°) indicates a fully methylated surface, effectively shielding the inorganic-like backbone[109].

Medical grade silicone is widely used as a biomaterial for different applications, including tubing, catheters, mammary implants, testicular implants, plastic reconstruction, encapsulation of electronic components and voice prostheses. Silicone voice prostheses are applied in throat cancer patients after laryngectomy[12], breast

prostheses[110,111], shunt valves for hydrocephalus[110], space-maker lead, insulator[110], temporomandibular joints[112], contact lenses, catheters[113], membranes for oxygenators, and adhesives[114]. Numerous studies have been conducted on toxicity, stability, tissue responses and oxygen permeability[115,116].

Silicone rubber exhibits good tissue compatibility but absorbs lipids from the blood and is thrombogenic (causes clotting).

Non-reactive silicones are mainly based on PDMS. For the long term application of the silicones used, the stability and the biocompatibility of the polymers are crucial. It is necessary however to try to improve the surface property of PDMS when being used as blood compatible material[117].

2.4.2 Vulcanization of Silicone Rubber

The curing mechanism of the PDMS shown in Figure 2.2 (below) has been fairly well established[118,119].

2.4.3 Mixing

Silicone rubber may be compounded in conventional equipment, such as dough mixers, Banburys and two-roll mills. The two-roll mill is useful for compounding silicone rubbers and is excellent for colouring, adding catalysts (for curing) and preparing primer stocks[11].

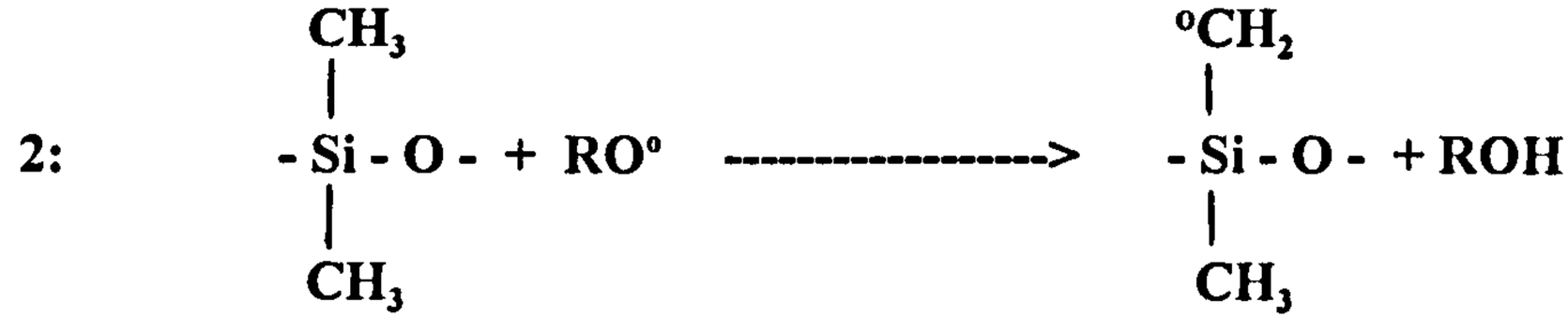


Figure 2.2 Peroxide cure mechanism for methyl siloxane.

2.4.4 Moulding

Compression and transfer moulding are the most widely used methods for moulding silicone rubber parts. However, large volume applications in the automotive industry are giving rise to a large growth in the use of injection moulding. Three mould variables that must be controlled are temperature, speed of mould closing and

pressure[11].

2.4.5 Extrusion

Gaskets, tubing, tape, wire and cable, seals, rods, channels and hose in a variety of shapes and sizes may be extruded. The equipment is similar to that used with other organic rubbers. When silicone rubber is extruded, however, the low green strength and the decomposition temperature of the peroxide curing agent must be considered. Typical extruders have screws with a length to diameter ratio of 10/1 to 12/1 although shorter screws are sometimes used[11].

2.4.6 Modification

Interest in using siloxane polymers has been steadily increasing because of their good biocompatibility, high gas permeability, unique optical and mechanical properties[13].

Ar- plasma treated and PHEMA grafted silicone rubber surfaces have been studied. The PDMS film, without plasma treatment, exhibits a water contact angle of 105°, indicating that the hydrophobicity of the polymeric films is drastically decreased by Ar-plasma treatment. The films treated with plasma but not yet grafted show a gradual increase in contact angle with time. PHEMA has been incorporated onto polymeric surfaces through the use of plasma induced graft copolymerization. The introduction of PHEMA onto a hydrophobic support has provided an adequate surface for rabbit corneal epithelial cells attachment and growth. At 72 h, a PHEMA grafted silicone

a PHEMA grafted TPX surface[30].

Techniques have been developed for radiation grafting monomers, such as NVP (vinylpyrrolidone) and HEMA, onto silicone rubber. This produces a composite material with a hydrophilic surface on a strong, stable backing[120,121].

NVP has been grafted under a variety of conditions onto silicone rubber tubes. For this purpose the silicone tubes have been subjected to cobalt-60 gamma radiation while swollen in NVP solution in toluene , the latter compound being used as a swelling agent for the silicone rubber, thus insuring a fast diffusion of the NVP monomer into the bulk of the tube walls[122].

An ammonia gaseous plasma modification technique was used in 1990 by Sipehia *et al.*[123] to modify polymeric surfaces, such as PMMA, PS and silicone rubber membranes. The purpose of this research was to study the enhancement of cell attachment and growth of rabbit corneal epithelial cells.

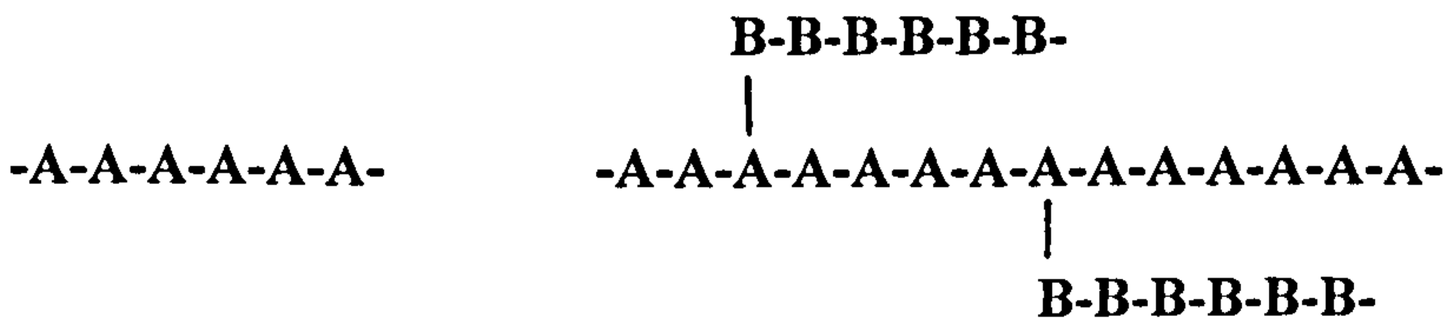
Hsiue *et-al.*[124] have modified the surface of silicone rubber by plasma deposition polymerization of HEMA. In this work the control and argon plasma treated and ppHEMA modified surfaces were used to assess the attachment and growth of rabbit corneal epithelium cells. They reported that the hydrophobicity of the treated silicone rubber films was drastically decreased[124].

Weathersby, *et al.*[125] and Hoffman[126] have found that the most blood compatible PDMS as hydrophobic surface could be prepared by radiation crosslinking pure (silica and catalyst free) PDMS in a nitrogen atmosphere. They concluded that PDMS had an intrinsically high thromboresistance if prepared in such a pure condition. Their results

intrinsically high thromboresistance if prepared in such a pure condition. Their results emphasize the importance of inert atmosphere and monomer and polymer purity, even when using "clean" radiation as a reaction initiator, for the preparation of biomaterials[126].

2.5 GRAFTING

While a homopolymer is made up of chains containing monomer units (species I) that are all alike, a graft copolymer is composed of chains containing two or more chemically different types of monomer units. If the block (-A-A-A-A-) and (-B-B-B-B-) are linked at one of their ends, a graft (II) or block copolymer (III,IV) can be formed. A graft copolymer, in the more generally accepted sense of the term, is made up of a backbone chain consisting entirely of monomer A, attached to one or more side chains composed of monomer B, as shown diagrammatically in the following example.



(I) (II)

-A-B-A-B-B-B-B-A- -A-A-A-B-B-B-

(III) (IV)

Homopolymers and random copolymers are relatively homogeneous in composition

throughout their bulk-graft copolymers and block copolymers, on the other hand, often demonstrate large compositional differences between surface and bulk and these differences can be observed over many molecular layers extending from the surface into the bulk. Figure 2.3 shows the structure and related systems of different block and graft copolymers. Each of the structures depicted in Figure 2.3 will generate surfaces which can differ substantially from the bulk, both chemically and morphologically[127,128].

Grafting technology has long been known in polymer chemistry, but the selective modification of a polymer surface by grafting is a fairly new technology. One of the reasons such graft copolymers are of interest to the polymer chemist is that a film or fibre of a chain of (A) can be surface grafted with branches of (B) to increase surface water repellency, dyeability, solvent resistance, light resistance, mildew resistance, *etc.*[128]

As is apparent, a terminal group which is reactive to the functional groups present on the substrate polymer surface is required for the polymer chains to be used for the coupling reaction, while the graft polymerization method needs active species on the substrate polymer to initiate radical polymerization[1].

When graft polymerization technology is applied for permanent surface coverage with water-soluble polymer chains, active species, such as trapped polymer radicals and peroxides, should be present in the surface region of the polymer substrate for the initiation of radical polymerization. In this case also, there are many methods to generate the active species. If the substrate polymer is cellulose, PVA or polyurethane,

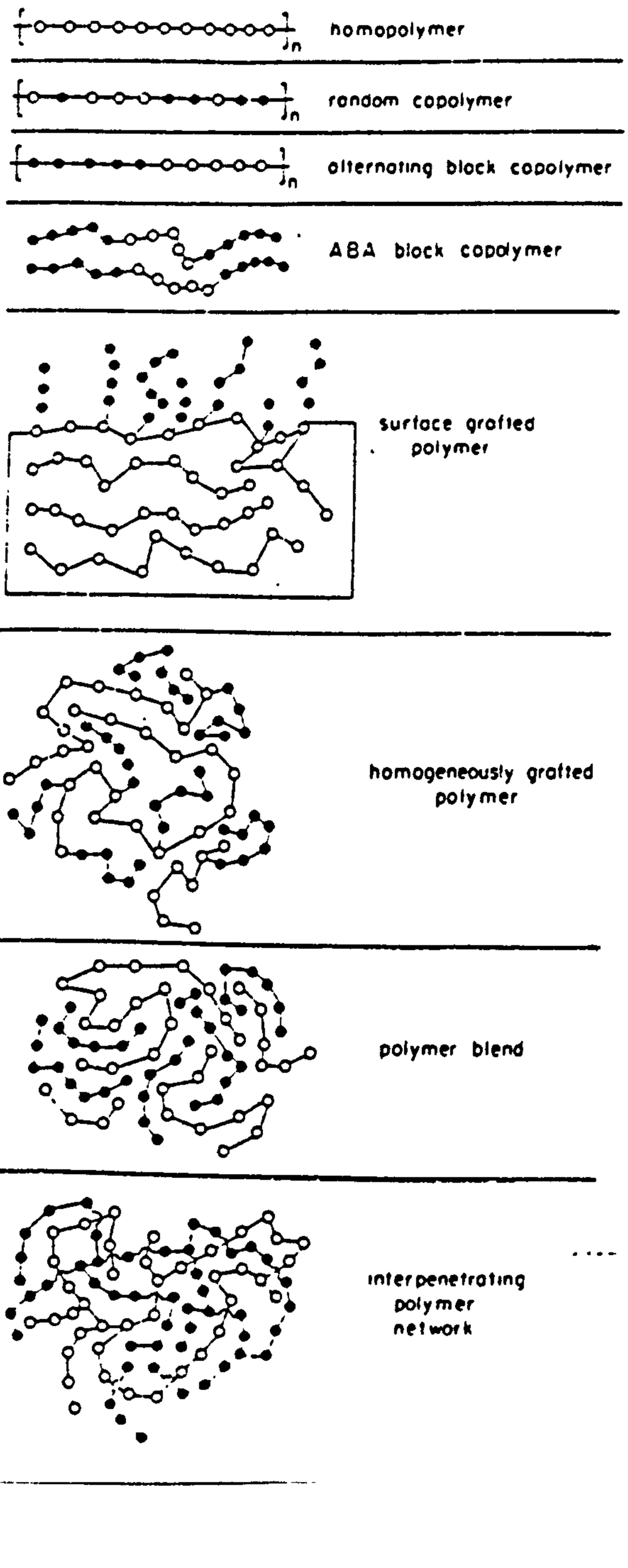


Figure 2.3 Structure and related system of different copolymers.

ceric ions can produce radicals on these polymer substrates. In contrast to the ceric ion method, which is applicable only to limited polymers, dry processes using ionizing radiation, UV, low temperature plasma, and ozone gas are effective for almost all polymer substrates, because these treatments generate, almost non-selectively, free radicals or peroxides on the treated surface, the density of the active species formed depends on the nature of the substrate polymer and on the treatment conditions[1].

2.5.1 Radiation Grafting

The use of high energy radiation for the synthesis of graft copolymers began to be explored early in the 1950's and has been investigated in great depth since that time. During the last 30 years, radiation chemistry has been, and still is, an active area of polymer research. High energy radiation *e.g.* electron beam and gamma radiation, initiates ionization and radical formation in polymers which may result in modification, crosslinking and / or degradation. An early approach, commonly modified, involves exposure to ionizing irradiation followed by free radical initiated graft copolymerization of monomers present or subsequently added[1]. Radiation induced free radical graft copolymerization has also been accomplished using UV light. In the work of Ranby *et al.*, surface photo grafting research has developed two processes applied to sheets, films and fibres of PE, PP, PET[129-131]. In principle there are two main methods of their synthesis.

1- Two polymer chains of different types can be directly combined by a crosslinking reaction. *i.e.*,

polymer A + polymer B --------> A-B graft copolymer

2- A polymeric backbone can have active sites created onto it, such as free radicals or ions. These are either simultaneously or consecutively reacted with a monomer or monomer mixture of different type to grow the grafted side chains, *i.e.*

polymer A --------> polymer A*

polymer A* + M_b -------> A-B graft copolymer

Where A* is a polymeric radical or ion[98,127,128.]

2.5.1.1 The Mutual or Direct Grafting Technique

Here the polymeric backbone is irradiated in the presence of the monomer or monomer mixture.

polymer p_a -------> $P^{\circ}_a + R^{\bullet}$

where P°_a is a macroradical and $R^{\bullet}$ a radical fragment such as H°, Cl°, CH_3, *etc.*

$P^{\circ}_a + M_b$ -------> P_a-P_b graft copolymer

eventually the growing graft or homopolymer chains terminate, either by combination or disproportionation.

To optimize the formation of graft copolymer with the minimum of contaminating homopolymer there are a number of conditions which need to be met[98,132].

There are methods of overcoming homopolymerization, for example a free radical inhibitor could be added to the monomer; Cu^{2+} or Fe^{2+} salts are examples which have been successfully used for the mutual grafting of acrylic acid to polyethylene[98,132].

An advantage of the mutual method is that most monomers act as radiation protectors reducing any degradation of polymer A by the radiation itself. Dose rate can also be an important variable[98,132].

Radiation induced grafting of dimethylaminoethylmethacrylate (DMAEM) on PTFE and PP films, and the preparation of heparin-modified materials from the resulting copolymers have been carried out in good yield[133]. The surface of polymer has been characterized for biocompatibility and for mechanical properties.

2.5.1.2 The Pre-Irradiation Grafting Technique

In this method the polymeric substrate is irradiated in the absence of air, subsequently the dearated monomer (vapour or liquid) is introduced into the vessel containing the irradiated polymer. The free radicals trapped in the polymer react with the monomer forming the graft copolymer. Very little homopolymer is produced by this method. A disadvantage of this method is that there is no protection of the substrate polymer by the monomer and some degradation can occur. The success of the technique depends largely on the crystallinity of the substrate polymer. Thus polymers such as PP with low crystallinity are particularly unsuitable for this technique. In this case the product would be a mixture of block and graft copolymer [98,128].

2.5.1.3 The Peroxide Method

This method can be illustrated schematically in the following way:

1- Polymer A + O_2 (air) -------> polymer A-OOH + some

polymer A-O-O-A polymer

2- Polymer AOOH -------> polymer AO° + $^{\circ}OH$

3- Polymer AOOA polymer --------> 2 polymer AO°

If stage 2 is carried out in the presence of monomer B, in the absence of oxygen, it is clear that grafting will occur.

In the case of 2, homopolymer will result from initiation of polymerization by the released $^{\circ}OH$ radicals. In general, with most polymers, hydroperoxides are by far the most prevalent and so homopolymer formation can become a problem. In the correct circumstances this can be alleviated by the use of a selective redox system.

e.g. **polymer AOOH + Fe^{2+} -------> polymer AO° + OH^{-} + Fe^{3+}**

In this way the production of homopolymers can be largely avoided.

The above discussion has, even in the case of mutual grafting, implied a heterogeneous reaction[98,128].

As reported, peroxides formed on the surface by corona treatment of low density PE film can be used to initiate grafting of polar vinyl monomers such as acrylic acid[20].

Iwata *et al.*[134] showed that it was possible to use the peroxides formed on a PE surface during corona treatment to initiate grafting of AAm in aqueous solution.

According to Fujimoto *et al.*[135] graft polymerization of AAm onto polyurethane (PU) was performed. Treatment on the polymer film was first carried out to introduce peroxides on the surface using a low temperature plasma generator; these were then decomposed thermally to yield polymer radicals which were capable of initiating

monomer polymerization. In the case of redox initiated polymerization, Mohr's salt [Fe $SO_4(NH_4)_2\ SO_4\ 6H_2O$] was added to the monomer solution[135].

Radiation grafting lends itself well to changing the location of the reaction. The added monomer can either be allowed to penetrate the whole substrate or only given brief contact (surface contact) before irradiating.

These two approaches would thus tend to give bulk or surface grafting respectively. As reported the swelling method applies equally to both the direct and pre-irradiation technique. Radiation induced graft copolymerization of AAm has been carried out onto films or tubes of PE and PVA in an attempt to make the surfaces blood compatible[136]. A series of studies regarding surface modification of polymeric films with graft polymerization has been started by Wang and co-workers, using a plasma technique[124]. The oxygen removal process may be disadvantageous when the photografting is commercially utilized as a method for functionalising polymer films on a large scale[137]. Recently, Uchida *et al.*[138], and Kupoota[139] have developed a new method of omitting the oxygen-removal procedures. Photografting of acrylamide onto the surface of PTFE film without a photoinitiator has been done, in which an appropriate quantity of periodide was added[139].

2.5.2 Properties of Ionizing Radiation

Strictly speaking, radiation consists of electromagnetic quanta or photons and ionizing radiation consists of high energy photons. In practice, ionizing radiation is a

term used not only for high energy photons, but also for energetically charged particles (electrons, protons, alphas, ionizing fission fragment, fast neutrons, *etc.*).

The use of radiation in grafting has several potential advantages over alternative methods. The yield of the radiation sites on the main chain is independent of temperature and the radicals are generated uniformly throughout the polymer to be modified at a rate proportional to the dose rate[140].

The introduction of polar substituents, *e.g.* as grafted chains onto a hydrophobic polymer surface is expected to give favourable modification of the polymer properties. Several methods have been used for this purpose, including X-ray and gamma ray and UV-light initiated grafting[139-147]. Every method has its merits and disadvantages. Photografting is not a new process. The pioneering work on polymer photochemistry was done by Oster *et al*[148,149]. Tazuke and Kimora[150] , have studied photografting of acrylamide on PP initiated by benzophenone. Nito *et al.*[144] have observed that acrylamide attained a high percent grafting (about 100%) applying the Tazuke and Kimura method to a stretched PP film. Hyakawa *et al.*[34] have reported graft copolymerization of maleimide onto ethylcellulose and PE films by using sublimation under X-ray or UV irradiation. Angel *et al.*[151] have studied the photografting on PP using benzoin ethylether as initiator, by which monomers such as styrene, MMA and 4-vinylpyridine can be grafted with high yield. Ogiwara *et-al.*[152] have studied a two-step method to photograft acrylamide onto PE film. It is not easy to apply any of these methods for practical applications however, because, *e.g.* the irradiation time in several methods is too long. The operation of these techniques can not proceed continuously

and the severe vacuum conditions required in some methods are expensive and difficult to obtain in large-scale plant[16].

Ionizing radiation processing offers unique advantages for preparing novel biomaterials[126].

About 30 years ago it was found that a high energy ionizing radiation both from isotope sources and from electron accelerators could be successfully used for the initiation of grafting. The advantages and disadvantages of these methods have been presented by Chapiro and Pekala[153,154].

2.6 APPLICATION OF SURFACE MODIFICATION

2.6.1 Wettability

Adamson[155] states that wetting means that the contact angle between a liquid and a solid is zero, or so close to zero that the liquid spreads easily over the solid surface, while non-wetting means that the angle is greater than 90°, so that the liquid tends to ball-up and run off the surface easily.

Thus there are basically two routes toward the modification of the wetting behaviour of a given material. The first involves the modification of the chemical composition of the material surface, in order to best exploit contact angle phenomena. The second which can of course, be coupled with the former, involves the macroscopic modification of the physical structure of the surface.

It is clear that most polymers fall in the region between the strongly hydrophobic materials poly(tetrafluoroethylene)(PTFE) and PDMS and water[156].

2.6.2 Hydrophilic Surfaces

The best way to improve hydrophilicity is to allow water to engage its preferred interactions with the solid, *i.e* short range hydration, acid-base and hydrogen bonding interactions. This can be done with surface modification techniques that introduce hydrophilic groups on the substrate surface. When it comes to hydrophobicity, not only must these interactions be prevented, but also the residual Van der Waals' interaction must be minimized. This is best done with the introduction of fully fluorinated or methyl groups[19].

Besides hydrocarbon based polymers, studies on the mechanism of hydrophobic recovery have been performed on siloxanes[24], fluoropolymers, polycarbonates, poly(ethyletherketone)(PEEK), and poly(ethyleneterephthalate)(PET)[157-160].

Very low contact angle surfaces require not only surface groups which can interact with water but also all shielding or short range hydrophobizing effects should be prevented. In order to obtain a very low (<15°) contact angle, not only must hydrophilic groups be present, but also the details of the molecular conformation on the surface must be favourable.

The surface modification of materials with grafted PAAm chains will be confirmed from the contact angle measurement which is one of the best method for assessing the hydrophilicity of surfaces[136].

2.6.3 Hydrophobic Surfaces

As shown by Figure 2.4 and by everyday experience, more common synthetic polymers are, in a general sense, hydrophobic, since a drop of water doesn't spread over their surfaces.

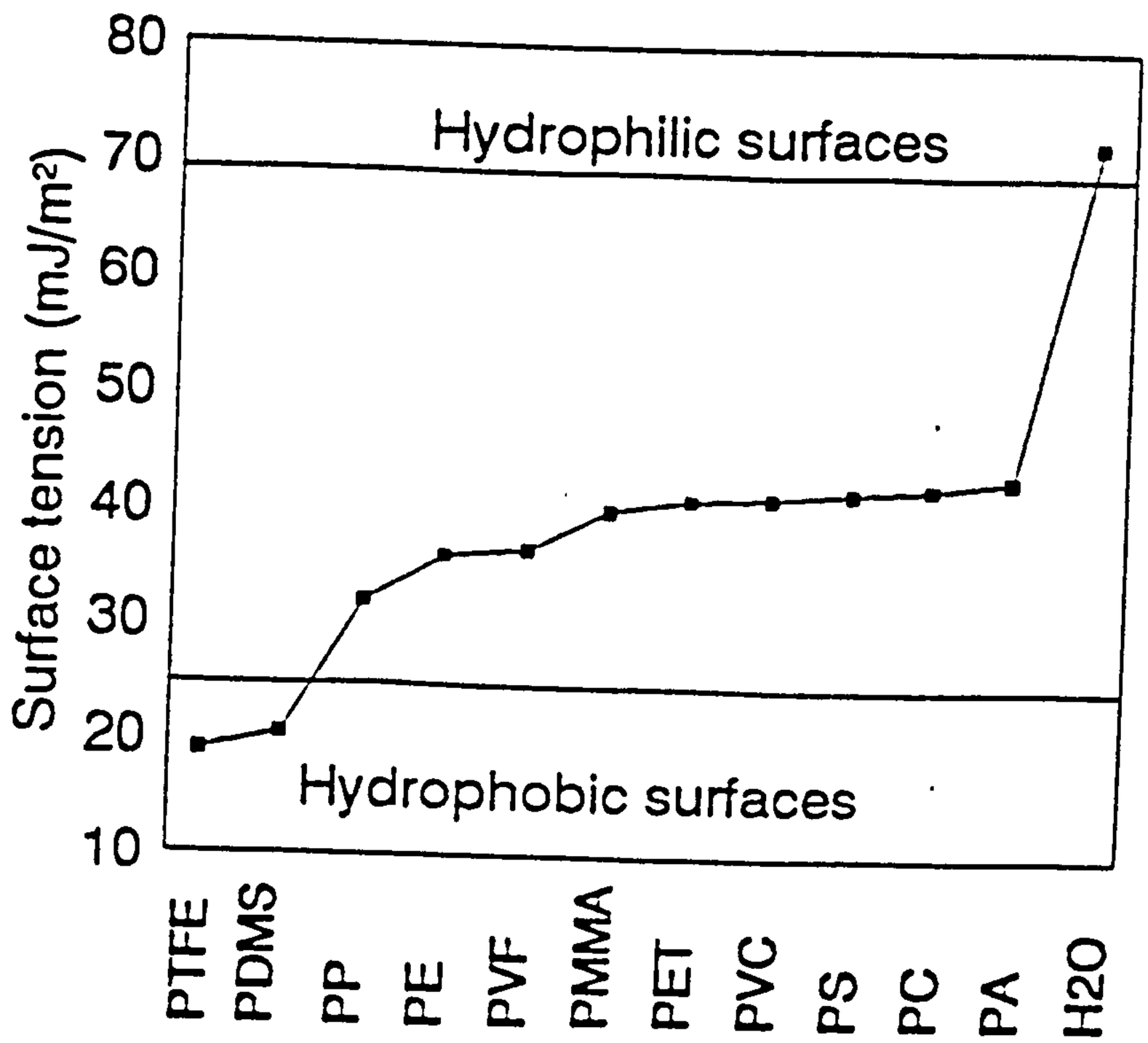


Figure 2.4 Surface tension of common polymers and water.

Many definitions of what is intended by hydrophobic surfaces exist, the most commonly quoted being, as stated above, that of a greater than 90° contact angle[155].

Measurement of the contact angle of water on a sample surface provides an estimate of the hydrophobicity of the surface. The higher the contact angle, the higher the hydrophobicity.

Advancing contact angle formed between distilled, deionized water and the two surfaces of the different types of PDMS films was measured at 25 °C and ambient atmospheric pressure.

The first requirement for making surfaces hydrophobic is to prevent water from establishing its special interactions. Thus, groups able to engage hydrogen bonding with water should not be present on hydrophobic surfaces. Such very high contact angles make drops of water roll on the surface as freely as mercury drops on glass[155].

It is believed that unexpectedly high contact angle phenomenon are related to both the microfibrous surface morphology of the polymer and its surface chemistry[2].

Weathersby *et-al.* have reported that the PDMS crosslinked samples as hydrophobic polymer system showed blood compatibility[125].

According to Ikada's calculations, there are two possibilities for non adhesive polymer surfaces in aqueous media (W_{12w}=0), that is (superhydrophobic) or (superhydrophilic) surfaces[14].

The reason for superhydrophobic behaviour is not completely clear. In principle, there is no reason why a hydrophobic interface should be created, but as shown by the other studies, events in surface treated polymers are controlled by a large number of

different interactions, both chemical and physical, involving modified chains and the environment, surface and subsurface molecules and interactions among the functional groups introduced by treatment.

In summary, the hydrophobization of polymer surfaces can exploit, from a strict surface physico-chemical point of view, two different structures; the first one is based on fluorinated groups, while the second one involves hydrocarbon groups[24,157,159,160,161].

A study by Morra *et-al.*[109] showed that oxygen-plasma treated PP aged in air at room temperature gave an increase in the advancing angle, suggesting that oxygen containing polar groups remain on the restructured surface.

2.7 BIOMEDICAL MATERIALS

2.7.1 Introduction to Biocompatibility

Most conventional materials do not meet the demands required for both their surfaces and bulk properties when used as biomaterials. An effective approach for developing a clinically applicable biomaterial is to modify the surface of the material which already has excellent biofunctionality and bulk properties[1].

The surface of biomaterials very often has a decisive influence on their performance because it can trigger foreign body reactions by getting proteins and cells attached to it. The concept of biocompatibility has long been debated but a widely approved conclusion on the term has not been achieved. The reason is simply because biocompatibility involves such diverse concepts as not to be concisely defined[162].

A biomaterial can exhibit toxicity when water-soluble or dispersible substances leach from the biomaterial into the body either through diffusion or biodegradation of the biomaterial. In contrast, biocompatibility is unrelated to such leachables but is inherent in the biomaterial itself. As illustrated in Figure 2.5, biocompatibility can be divided into two constituent parts. One is concerned with the bulk property of the biomaterial, the other with its surface property. The bulk biocompatibility is very influential when the biomaterial is implanted either under direct bonding with a living tissue such as blood vessels and bones or under high loading[1].

Interfacial biocompatibility is closely related to the events occurring at the interface between the biomaterial and the living cell or tissue. The purpose of surface modification is to improve this kind of biocompatibility for the biomaterial which is not toxic and possesses good bulk biocompatibilty[1].

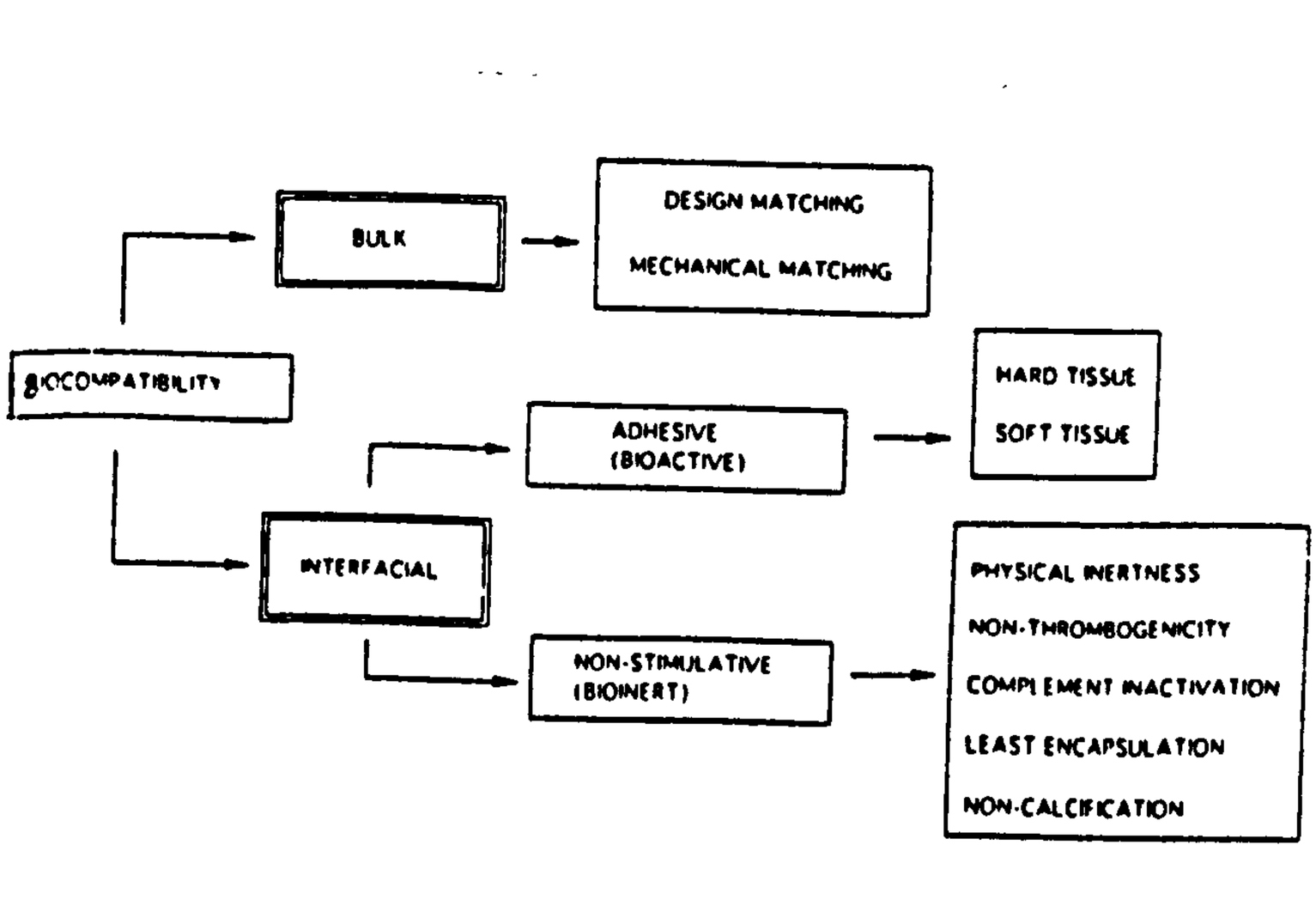


Figure 2.5 Bulk and interfacial biocompatibilities.

It is the surface of a biomaterial which first comes into contact with the living body when the biomaterial is placed in the body or exposed to fresh blood. Therefore the initial response of the living body to the biomaterial must depend on its surface properties. If the biological response towards the foreign body (foreign body reaction) is very strong, the biomaterial will be markedly disturbed to perform its function. For instance, a haemodialyser will not work well if a clot is formed on the haemodialysor surface[1].

As is obvious from the above example, commonly used materials do not always possess the surface properties necessary for biomaterials. It follows that most conventional materials need surface modification if they are to be employed as biomaterials. It is rather strange that industrial materials have been applied for a very long time in medicine without any surface modification but simply after purification to exclude toxic substances from the materials. The next generation of biomaterials to be used clinically should have excellent properties both in bulk and on its surface. At present it is very rare that a material with good bulk properties also possesses the surface characteristics required for the biomaterial. This is the major reason why surface modification is in many cases essential for a material to be applied in medicine.

2.7.2 Tissue Compatibility

When a biomaterial is implanted in the body, it is phagocytosed by macrophages if the size is much smaller than the cell. If it is too large for the macrophages to phagocytose, macrophages adhere on the surface. This macrophage reaction is the

initial biological response to the implanted foreign material. As a result of this macrophage attachment, a series of foreign body reactions start, resulting in encapsulation of the implanted material by collagenous connective tissue. The phagocytotic uptake and adhesion of macrophages greatly depends on the surface properties of the biomaterial, in addition to its geometrical size. Uptake by phagocytotic cells and material encapsulation by collagenous tissue have to be avoided; surface modification of the material is needed to render it bioinert.

The lipophilization was performed by alkylation of the cellulose surface with different alkyl amines [163]. As can be seen, the uptake dependence on the surface hydrophilicity/ hydrophobicity balance is similar to that observed for cell adhesion onto different surfaces. Macrophage uptake is greatly reduced if the material surface is extremely hydrophilic or hydrophobic, whereas the microsphere uptake is markedly enhanced if its surface hydrophilicity or hydrophobicity is intermediate. As pointed out earlier, an effective method for surface modification to make the material surface least attractive to macrophages, *i.e.* bioinert, is to cover the surface with non-ionic, water soluble chains. In contrast, coverage of the material surface with opsonic proteins such as collagen, immunoglobulins and fibronectin will greatly promote macrophage uptake. Such surface modification is very important, especially for microspheres to be used for drug delivery systems. Generally, microspheres administered into the body are captured by the phagocytotic kupffer cells in the liver before achieving their function in the body. Rapid phagocytosis is strongly required when immunopotentiation by macrophages has to be enhanced[1,163].

The major surface properties that should be modified include two kinds of biocompatibility, one is the surface property that elicits the least foreign body reactions and the other is the cell and tissue bonding capability[1].

Cell attachment and growth onto surfaces of (PHEMA grafted silicone rubber) have been examined by light microscopy[30].

Hydrophilic surfaces are more suitable for cells, however, and both hydrogels such as agar (a polysaccharide) and poly(HEMA) have adequate biocompatibility and have found many applications where cell adhesion is not desirable. Graft polymers have a number of important advantages for usage involving modification of polymeric surfaces. Thus, by covalently bonding a polymeric chain onto the surface of other polymers, a novel surface is formed[30,164].

As already reported by Rajarman *et al.* the change in the morphology of a fibroblast cell during adhesion and growth on a substrate *in vitro* goes through four stages; attachment, growth of filopodia, cytoplasmic webbing and flattening (Figure 2.6)[165,166].

It has been found that the extent of cellular adhesion is considerably affected by physical and chemical properties of the substrate surface, *i.e.* chemical composition, charge, hydrophilic-hydrophobic balance, microstructure, rigidity and adsorbed protein layer[166,167].

As reported, tissue compatibility was estimated from the thickness of the fibrous tissue capsule formed around implanted test specimens in the back region of rats[168].

Biological performance will depend not only on the hydrophilicity of the system but on numerous other parameters including the chemical composition, types and number of

crosslinks, presence of functional groups, quasi-organized water structure, porosity, and the thermodynamic interaction parameters between these components of the biological environment and the gel[169].

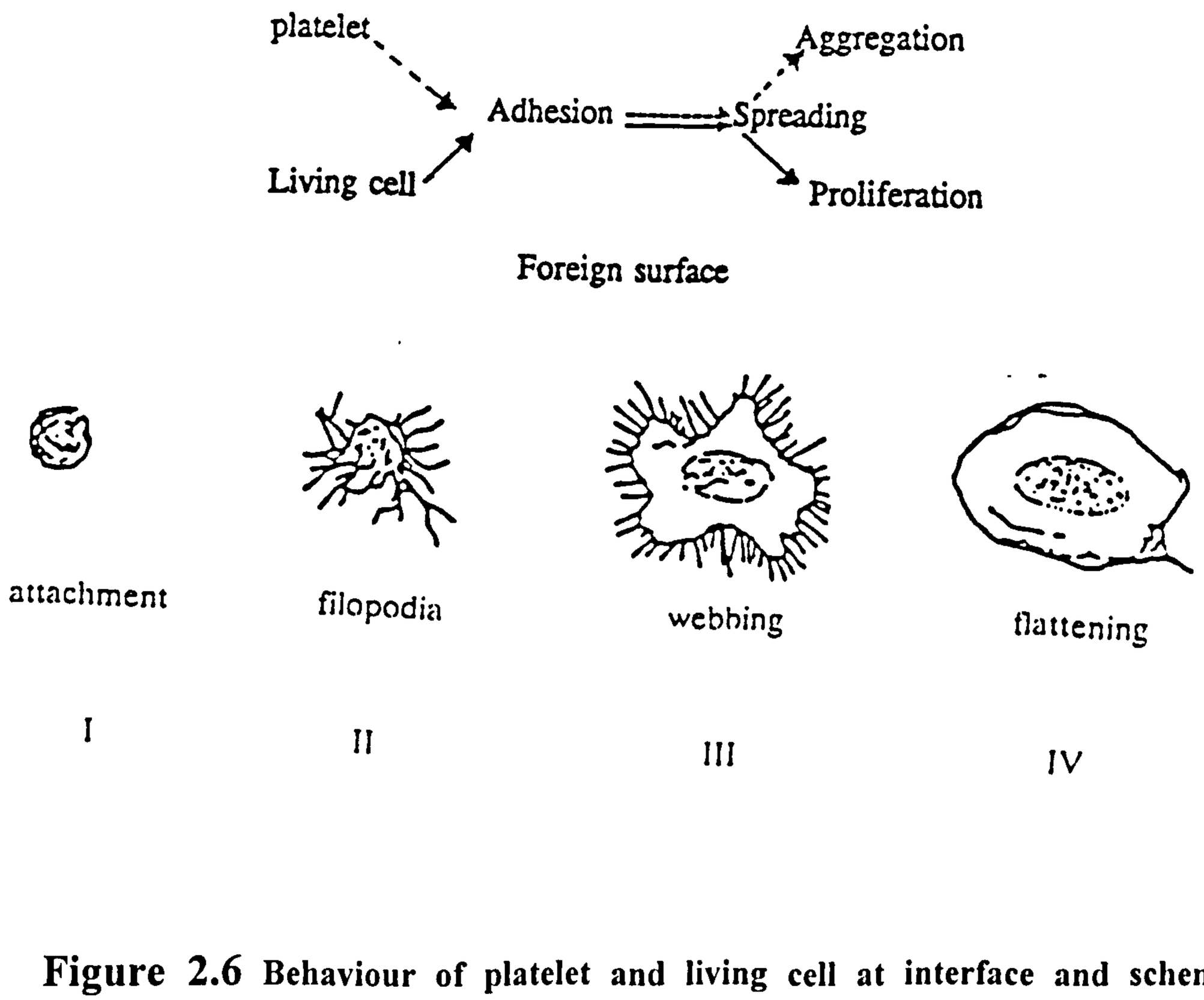


Figure 2.6 Behaviour of platelet and living cell at interface and schematic presentation of different stages in adhesion and spreading of a fibroblast cell.

2.7.3 Blood Compatibility

One of the most important biocompatibilities of biomaterials is blood compatibility. Since platelet aggregation and fibrin network formation take place sooner or later when a conventional foreign surface is brought into contact with fresh blood, a large number of methods have been proposed for rendering the material surface blood compatible[1].

Biomaterials which come into direct contact with blood are used for short term and long term applications, as shown in Table 2.3.

If the period of contact with the flowing fresh blood is short, up to several hours, as in extracorporeal circulation, heparin, an anticoagulant glycosaminologyean, is in most

Table 2.3 Methods for blood compatibility.

Blood contact	Method
Short- term	Calcium ion removal Heparinized blood
	Antithrombogenic surface
Long- term	Neo-intima formation (cell
	seeding)(antithrombogenic surface)

cases administrated to the blood to prevent blood coagulation. When blood is stored in a container unit before the clinical test or transfusion, chelating agents are added to the

blood to remove calcium ions.

If blood contact with a material surface continues as long as several days or weeks, slow release of heparin from the device or immobilization of fibrolytic enzymes on the surface occurs. This kind of treatment has been applied to the catheters used for intravenous hyperalimentation. No effective methods have yet been developed for surfaces which are permanently in contact with flowing blood. The medical devices for such long term applications include artificial hearts, blood vessels and heart valves[1]. It is well known that thrombus formation is triggered by an interaction between blood components and the foreign polymer surface[170].

It is not recommended to administer high concentrations of heparin to patients even if the duration of blood contact is as short as a few hours, because any haemostatic mechanism does not control internal or external bleeding. As a consequence, a very large number of investigations have been directed to the synthesis of antithrombogenic surfaces which do not need any anticoagulant.

As a consequence of this limitation only a few medical devices having a relatively antithrombogenic surface have been developed and are currently used in clinics and only for short term applications[1,171].

Solving this problem has been given an impetus by the increased clinical utilization of biomaterials in applications involving contact with blood, and there is a general belief that enhanced knowledge of the blood response to biomaterials is relevant for both well established procedures, such as the artificial kidney, and for procedures yet to achieve clinical acceptance, such as for artificial hearts[172,173]. Vascular surgeons have had to

make do with less than perfect materials, for example Gortex, an expanded PTFE material, has been used for over 20 years[2] but suffers from giving rise to formation of clots as well as having a poor tissue compatibility, resulting in dislocation of the implants.

It has been reported that pore size and porosity are very important in the design and production of synthetic vascular grafts[2,154,174].

Extensive studies on the blood compatibility of polymeric materials have revealed that it is strongly governed by their surface structure and properties. Among them are roughness, porosity, hydrophobic, hydrophilic balance, ionic species, and water content in the surface layer[2,136,174].

It has been reported that the role of complement activation in thrombus formation within implanted materials, although many medical studies that platelets, leucocytes, coagulation and the complement system may interact in blood[175].

Although there is still some controversy with respect to the optimum surface texture required for attainment of excellent thromboresistance, there have been reported a number of papers which support the hypothesis that a hydrophilic material with a high water content, that is, hydrogels, exhibit good blood compatibility[136]. However, according to Ratner *et al.*[15] the higher the water content of hydrogel materials, the more damaging the surface is to flowing blood. This finding is contradictory to the hypothesis of other authors as to what relationships might exist between hydrogel water content and blood compatibility. Their results indicated that hydrogels may be unexpectedly thrombogenic.

Figure 2.7 illustrates the postulated model of the AAm grafted outermost layer in contact with an aqueous protein solution. As shown in this figure the protein adsorption and platelet adhesion are greatly reduced by the surface graft polymerization of AAm, as long as the density remains low[135].

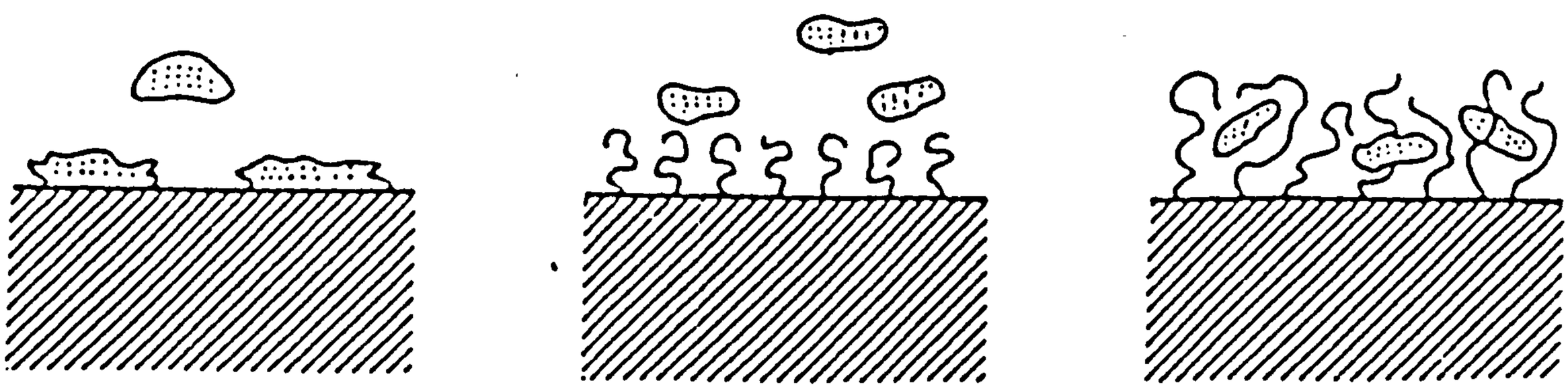


Figure 2.7 Schematic representation of grafted surfaces interacting with proteins a) non-grafted; b) grafted; c) excessively grafted.

The grafted polymer chains may prevent the protein molecules and platelet cells from direct contact with the PU surface owing to their steric hindrance effect. It has been reported that the pore size and porosity are very important in the design and production of synthetic vascular grafts [2,176].

One of the main problems associated with blood contacting medical devices is surface-induced platelet adhesion and subsequent thrombus formation. It is generally accepted that the thrombus formation is mediated by thrombogenic proteins, such as fibrinogen or fibronectin, which adsorb to the artificial surface and enhance the interaction of the surface with platelets. The presence of other proteins, such as albumin, on the surface is believed to inhibit adsorption of thrombogenic proteins to the surface and decrease platelet adhesion and activation[177].

Figure 2.8 clearly demonstrates that there are two possibilities for a polymer surface to have $W_{12,w}$ of zero, in other words, to be nonadhesive. One is to create a superhydrophilic, in other words, water-like surface (γ_{1w}=0) and the other, a superhydrophobic one (γ_{1w}=73)(erg cm^{-2}). This is an answer to the old and still debated question why two extreme surfaces totally different to each other are relatively blood compatible. Of these two possibilities the hydrophilic surface appears to be a more promising candidate for a long term blood compatible polymer. Ikada *et al.* have reported that it is almost impossible to synthesize the hydrophobic polymer which exhibits higher γ_{1w} than perfluoropolymers possessing the lowest γ_1 among the conventional polymers. On the contrary, it may be much easier to modify the polymer surface so as to have a $W_{12,w}$ close to zero[14]. They have derived an expression for the

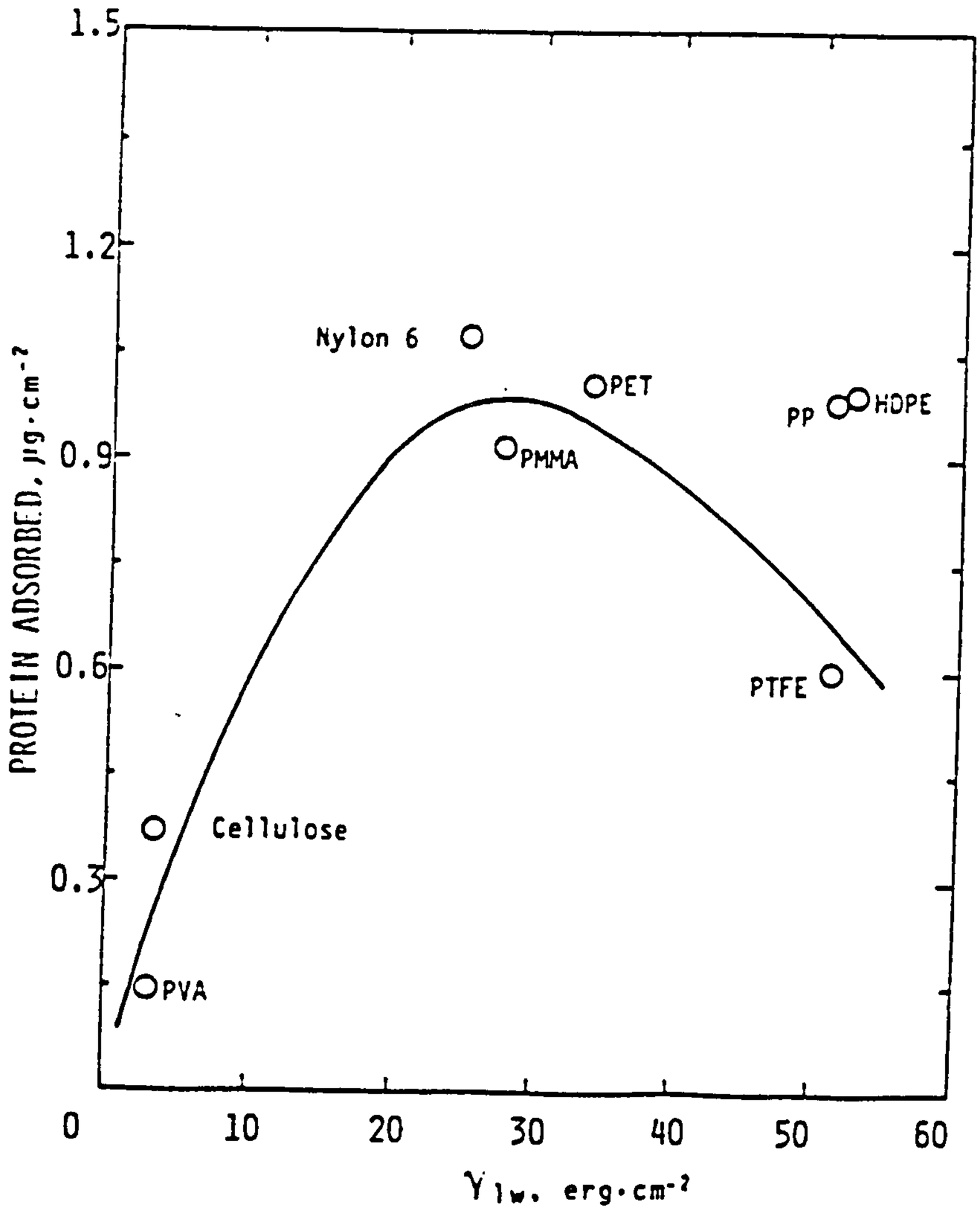


Figure 2.8 **BSA adsorption to different surfaces at 37 °C in phosphate buffered solution. The initial BSA concentration is 3 mg.ml^{-1}.**

work of adhesion of polymer material 1 with adherent 2 in a medium of water ($W_{12,w}$). When the $W_{12,w}$ values calculated for many conventional polymers from the derived equation were plotted against the interfacial free energy between material 1 and water

(γ_{1w}), they obtained a curve having a maximum, indicating $W_{12,w}$ to approach zero if γ_{1w} is either zero or equal to γ_w. This suggests that the surface, either superhydrophilic (γ_{1w}=0) or superhydrophobic (γ_{1w}=γ_w) may possess excellent blood compatibility. It is almost impossible at present to develop such a material that has a higher γ_{1w} at present than perfluoro polymers, whereas the surface having γ_{1w} close to zero may be more readily prepared by binding a water soluble polymer layer on a material surface. For instance, protein adsorption onto a PE film is decreased when AAm is graft copolymerized onto the film surface[14].

Rucinska *et al.*[178] have studied the reconstruction of blood vessels, one of the most rapidly developing trends in recent surgery. For many years, investigations have been carried out in order to find a substitute material which, after implantation, has the following properties 1) it has a small diameter and retains appropriate potency; 2) it does not undergo lateral twisting at the place of flexing; 3) it is infection resistant; 4) it is not susceptible to the forming of aneurisms and wall hypertrophy; 5) it has a stable internal surface which does not favour thrombosis. None of the recently available grafts and vascular prostheses has all of these desired properties[178].

The clotting times, in general, did not correlate well with the contact angle results or the level of fibrinogen adsorption. When blood contacts a foreign surface, for example, adsorption of serum protein occurs at first, and the adhesion, deformation and release reaction of platelets occur. From the viewpoint of blood compatibility, strong interaction between the material and platelets is undesirable[166].

2.7.4 Lubricious Surface

PDMS is not wetted by saliva and an inadequate lubricating film is formed, resulting in frictional problems. Such frictional effects may be expected to cause patient discomfort. Abrasion is due to frictional phenomena between human skin and prostheses and can be observed when a portion of tissue moves under a stable prosthesis or shifting prostheses move over stable tissue. The modification of the surface topography(smooth) or surface free energy (wettability) might combat the frictional effects between intraoral and extraoral prostheses and mucous membrane or skin[179-181].

Tubular medical devices such as catheters, cannulae, endoscopes and cystoscopes are inserted into the body orifices open to the outside. If there is a significant frictional resistance between the device surface and the mucous membrane of the body during the insertion or removal of the device, mechanical damage will occur on the mucous membrane. An effective method to reduce this friction is to make the surfaces of the device very lubricious, similar to the surface of mucous membranes. This is readily accomplished by graft polymerization of non-ionic water soluble monomers onto the device surface to a high level[1,182,183].

Guide wires with a very slippery surface are currently available for angiography. They do not come into direct contact with a living surface but with the inner wall of a catheter. The lubricious surface of the guidewires can be provided by both coupling reaction of a water soluble polymer and graft polymerization[184].

Hydrogel coating has been tried to make the surface of catheters very slippery to

reduce the frictional resistance between the catheter surface and the mucous tissue[1]. However, the aim is modifying surfaces by covalent bonding without any bulk deterioration and to reduce the cost of producing such biomaterials while at present is very high[1,185].

2.8 POLYMERS AND MEDICAL APPLICATIONS

2.8.1 Introduction

The types of synthetic polymers needed for biomedical application can be grouped roughly into three categories: (1) polymers that are sufficiently biostable to allow their long-term use in artificial organs, blood pumps, blood vessel prostheses, heart valves, skeletal joints, kidney prostheses, and so on: (2) polymers that are bioerodable-materials that will serve a short- term purpose in the body and then decompose to small molecules that can be metabolized or excreted, sometimes with the concurrent release of drug molecules: and (3) water - soluble polymers (usually bioerodable) that form part of plasma or whole blood substitute solutions, which function as macromolecular drugs. In general, the largest amount of effort has been evident for polymers of types 1 and 2, but materials in category 3 are of general interest as improved pharmaceuticals[186].

2.8.2 Heart Valves and Vascular Prostheses

Diseased aortic and mitral valves were first replaced with mechanical ball and cage prostheses in 1960. These devices have since been widely used. Some of the polymers employed are a Dacron cloth cuff and a silicone rubber ball. The housing of pacemakers contain epoxy resins. Silicone rubbers have been used in the electrical conduits of pacemakers[187].

Damaged heart valves, weakened arterial walls, and blocked arteries constitute some of the commonest cardiovascular disorders, and polymers. Defective heart valves can be replaced by mechanical valves based on various designs. A more recent design makes use of a small circular plate as a flap valve, with the flap made from pyrolytic carbon or poly(oxymethylene). Another surgical practice is to implant modified (crosslinked) tissue heart valves from pigs (porcine valves). Devices fabricated from synthetic hydrogels may eventually replace porcine valves[2,186]. Aneurisms (balloon-like expansions of the arterial wall) can be repaired by reinforcement of the artery with a tube of woven polyester or PTFE fabric; completely blocked arterial sections are removed and replaced by a tube of porous PTFE (Gore Tex). The polymer is relatively noninteractive with blood and its porosity favours the growth and anchoring of a lining of endothelial cells that insulate the blood from the polymer. Failure of such blood vessel prostheses usually occur by thrombus build up at the points of connections to the natural vessel. Evidence exists that this is partly due to the different elasticity of the natural vessel and the polymer, which induce fluid turbulence and deposition of blood platelets and thrombin. A clear need exists for new biopolymers that have an elasticity

comparable to that of the living tissue[2].

Polycarbonates have also found applications in heart / lung assist devices[187].

The clinical use of vascular grafts is in the main restricted to internal diameters larger than 6 mm. Problems of thrombus formation limit the use of smaller grafts. The polymers most often used are polyester fibre (Dacron) or PTFE, in knitted or woven form. The highly porous PTFE Gore-Tex allows ready formation of a new lining; vascular prostheses of 6 mm diameter are used in artificial kidney patients. Gore - Tex vascular grafts 4 mm in diameter have been used in coronary-artery bypass surgery. An expanded PTFE material called `impra graft' gives similar results[187].

2.8.3.1 The Artificial Heart

A considerable amount of research has been devoted to the design and testing of artificial heart pumps. Synthetic elastomers and rigid polymers have been used extensively for the construction of these devices. Unfortunately, most synthetic polymers accelerate the clotting of blood. This problem is so serious that animals on which the pumps are tested sometimes die within hours from the massive, gelatinous blood clots that form in the pumps. Avoidance of the clotting process is a complex problem because it depends on the design of the pump and the presence or absence of turbulence as well as on the materials used for construction[186].

2.8.3.2 Heart Pump Designs

Two types of pumps have been developed on an experimental basis: (1) auxiliary

blood pumps to by pass or supplement the action of a damaged heart until it can repair itself and, (2), total artificial heart pumps that can completely replace the living organ. Many of the booster pumps have used a rigid housing, often made of reinforced epoxy resin, with an internal tube of silicone rubber (Figure 2.9).

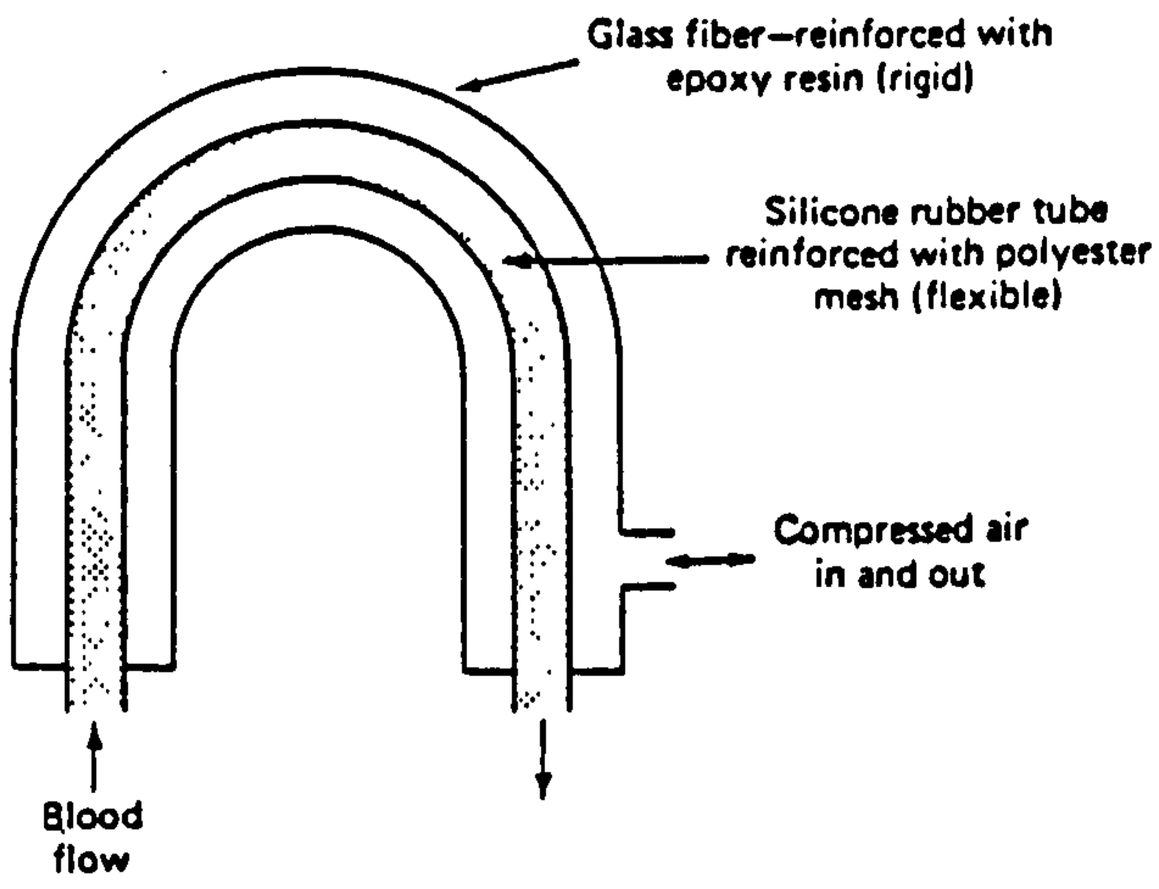


Figure 2.9 Relatively simple artificial heart device designed for implantation in the body. Pulses of compressed air compress the silicone-rubber inner tube, which is connected to the aorta. The phase of the pumping cycle is synchronized with that of the patient's heart[186].

Compressed air applied inside the rigid casing compresses the silicone or polyurethane rubber inner tube and this forces blood from the pump. A related device is the intraaortic balloon , a 25 cm x 2 cm polyurethane balloon inserted into the aorta which expands as compressed helium or carbon dioxide is pulsed in or out. Other devices use hemispheres of titanium, polycarbonate, or PMMA containing a PU diaphragm. Pulses of compressed air or carbon dioxide actuate the diaphragm and cause the pumping of the blood (Figure 2.10). Total artificial hearts have been constructed which resemble the general structure of a human heart but which are actuated by compressed gas or oil[186].

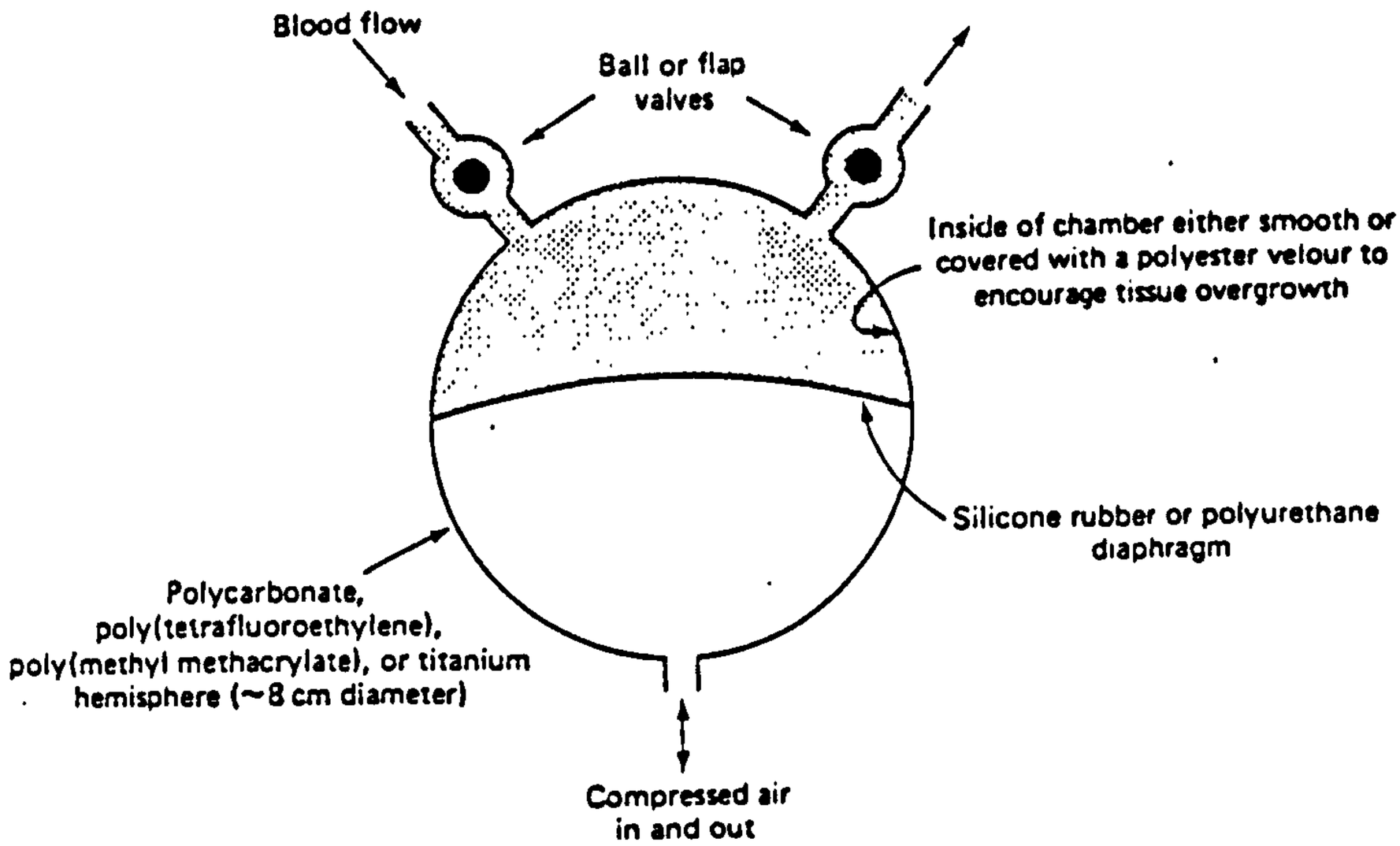


Figure 2.10 Schematic design of a hemispherical "artificial heart" pump, designed to operate outside the patient's body. The polyurethane rubber diaphragm is actuated by compressed air. Turbulence of the blood as it passes through the valves is a major cause of clotting[186].

2.8.3.3 Polymers For Heart Pumps

A wide variety of different polymers have been used for the fabrication of heart pumps. These include silicone rubber, PU rubber, Dacron polyester, Teflon, polycarbonate, PMMA, PVC, and pyrolytic carbon. The ability of a synthetic polymer to initiate the clotting of blood depends on the nature of the surface and on the chemical and physical properties of the polymer. For example, highly water repellent polymers appear to be among the best material for contact with blood[186,187].

2.8.4 Tissue Adhesives

The α-cyanoacrylate esters are the best known tissue adhesives. Methyl 2-cyanoacrylate proved excessively histotoxic and did not wet or readily spread on tissue. Isobutyl α-cyanoacrylate is much less toxic and retains good adhesive properties. A group of polymers based on the poly(α-cyanoacrylate) structure have proved to be effective for this purpose[186].

2.8.5 Artificial Skin

Several polymeric materials including reconstituted collagen have also been tried as burn dressings. Among them are the copolymers of vinyl chloride and acetate and methyl-2-cyanoacrylate. The methyl-2-cyanoacrylate was found to be too brittle and histotoxic for use as burn dressing. The ingrowth of tissue into the pores of spun (Ivalon, polyvinylalcohol), and woven fabric (nylon and silicone rubber velour) was also attempted. Plastic tapes have sometimes been used to hold skin grafts during

microtoning (ultra thin sectioning) and grafting procedures. For severe burns the immersion of the patient into silicone fluid was found to be beneficial for prevention of early fluid loss, decubitus ulcers, and reduction of pain[13].

The search for polymeric materials that can be used as synthetic skin to cover large burns has led to the use of synthetic poly(amino acid) films. For this purpose velours of nylon fibre have also been tested for this use, as have films of poly(α - cyanoacrylates)[186]. Many polymeric membranes have been investigated in the treatment of burns. These include velour fabrics (nylon or Dacron, PP, and rayon) backed with silicone rubber, poly HEMA polymerized in situ, block copolymers of propylene and ethylene oxides (pluronic F-127), and porous segmented polyurethanes. Synthetic polymers have not yet provided a skin substitute. Problems of infection, tissue compatibility, vascularization, and epithelization still persist. Cross-linked collagen-glycosaminoglycan coprecipitate is a desirable base material[188].

2.8.6 Bones, Joints, and Teeth

Bone fractures are occasionally repaired with the use of PU, epoxy resins, and rapid-curing vinyl resins. Silicone rubber rods and closed-cell sponges have been used as replacement finger and wrist joints, vinyl polymers and nylon have been investigated as replacement wrist bones or elbow joints. Furthermore, cellophane and, more recently, silicone rubber have been used in knee joints to prevent fusion of the bones. Dramatic advances have been made in hip-joint surgery with the use of stainless steel or PE balljoints attached to the femur by means of a PMMU filler as binder. Teflon

add to abbreviation list

fabric and silicone rubber have been used to make synthetic ligaments and tendons[13,187]. PMMU is the principal polymer used both for acrylic teeth and for the base material. Acrylic resins are also used for dental crowns, and epoxy resins are sometimes employed to cement crowns to the tooth post[187].

The ultra-high molecular weight PE (> $2x10^6$ g/mol) has been used extensively for orthopaedic implant fabrications, especially for load-bearing surfaces such as total hip and knee joints[13]. PP has an exceptionally high flex life and hence was tested in integrally moulded hinges for finger joint prostheses[13,187]. Bone cement has been used for clinical applications to secure a firm fixation of joint prostheses for hip and knee joints. Bone cement is primarily made of PMMU powder and monomer methyl methacrylate liquid. Ultrahigh molecular weight PE (UHMWPE) is used for a cetabulum cups in total artificial hips (TAH) and as the gliding parts in knee, shoulder, ankle, and elbow joints. PP or silicone rubber are used in finger joints[187].

The most commonly used bone cement is an acrylic resin self curing at room temperature. Artificial tendons made of PE have been investigated as well as a substitute collateral ligament[187].

2.8.7 Artificial Kidney and Hemodialysis Material

Cellophane (regenerated cellulose) has been used for semipermeable dialysis membranes in conventional kidney machines. In one particular development, a bundle of 2000 to 11000 hollow fibres of modified polyacrylonitrile (17 cm long and 300 μm diameter) are used. The polymer is heparinized to prevent blood clotting. Hollow rayon

fibres or polycarbonate or cellulose acetate fibres have also been used for the same purpose[186].

Commercial dialysis membranes are made of cellulose acetate, regenerated cellulose (Cuprophane), polycarbonate, PMMA, polyacrylonitryle, ethylenevinylalcohol copolymer (EVA), and polysulfone. They are used in disposable forms, *e.g.* as hollow fibres or integrated plates[187,189].

In peritoneal dialysis, the patient's peritoneum is used as a dialysis membrane; CAPD keeps the dialysate a longer time in the peritoneum. This procedure was initiated in 1975 using a silicone rubber, Tenckhoff indwelling tube and PVC plastic bag for the dialysate[187].

2.8.8 Oxygen Transport Polymers

PDMS membranes are highly efficient gas transporters. Silicone rubber membranes have also been tested in artificial gills for underwater breathing. It is of interest that silicone rubber has approximately six times the oxygen permeability of fluorosilicones, nearly 80 times the value for PE, and 150,000 times the permeability of Teflon. This could be connected with the high torsional mobility of the siloxane chains[186].

In open-heart operations, the heart is temporarily replaced with an extracorporeal heart-lung machine. This equipment is now a standard commercial product. In the bubble - type oxygenator, blood is directly exposed to oxygen. The oxygenating section can be made of soft PVC film or rigid polycarbonate. In the membrane-type

oxygenator, the blood is oxygenated through a polymeric membrane, *e.g.* PP, silicone rubber, PTFE or polysulfone[187].

2.8.9 Surgical Sutures

A synthetic replacement for catgut is synthetic polyglycolic acid. Polyglycolic acid has a high tensile strength and is compatible with human tissue. The polymer degrades by hydrolysis to nontoxic glycolic acid[187].

PP (Prolene and Vieryl), Dacron (Mersilene) and PTFE-coated PET (Tevdel) are typical nonabsorbable sutures. Nylon is not stable indefinitely in the body. The nylons are hygroscopic and lose their strength *in vivo* when implanted. The water molecules serve as plasticizers that attack the amorphous region. Proteolytic enzymes also aid hydrolysis by attacking the amide group[13].

2.8.10 Polymeric Blood Substitutes

There is a serious need for the development of a water soluble or hydrophilic polymer that is nontoxic and biodegradable. Some water soluble polyphosphazenes may be of value for this application. An emulsion of PTFE particles (less than 0.001 mm in diameter) or liquid fluorocarbons in water, together with glucose, salts, and surfactants, has been used to replace the blood of rats. The long range possibility also exists that biodegradable, water soluble macromolecules can be synthesized that possess oxygen carrying side groups such as metalloporphyrins[186].

2.8.11 Contact Lenses and Intraocular Lenses

Although rigid polymers such as PMMU have traditionally been used for hard contact lenses, the modern tendency is toward flexible or soft contact lenses. A soft contact lens is made from a lightly crosslinked, water-soluble polymer. Hydrogel research is an important area in many fields of biomedicine because hydrogels can be designed to mimic the physical character of many tissues such a cartilage, skin, blood vessel lining, *etc.*[124,186].

2.8.11.1 Hard Lenses

Contact lenses range from hard to soft: Conventional hard lenses contain no less than 95% PMMU. They are designed and fit in such a manner that the tear fluid supplies the oxygen required by the cornea. Hard and semirigid lenses permeable to oxygen have been developed, most of them are made from copolymers of siloxanes and methacrylates. Flexible, oxygen-permeable silicone lenses are also available[187-190]. Polymeric materials that have been evaluated for use in hard contact lenses include poly 4-methyl-1-pentene(TPX), cellulose acetate-butyrate(CAB) fluorocarbon polymers, and silicone-acrylate copolymers. All of these materials exhibit higher oxygen permeability than PMMU. The most widely distributed materials for high gas-permeable lenses are copolymers of 3-[3,3,3-trimethyl-1,1-bis(trimethylsilyloxy)disiloxanyl]propylmethacrylate[187].

2.8.11.2 Flexible Lenses

Hydrophobic flexible lenses manufactured from siloxane rubber are highly permeable to oxygen and can maintain normal respiration on the cornea surface without tear exchange under the lens[187].

2.8.12 Neurosurgery

Hydrocephalus shunts: these devices correct congenital hydrocephalus in children or infants. These prostheses use a valve connected to silicone rubber tubes which allow the cerebrospinal fluid to drain from the brain ventricle to the vena cava, atrium, or peritoneal cavity[187].

2.8.13 Oral and Maxillofacial Applications

In rhinoplasty and reconstruction of the external ear, silicone rubber is probably the most widely used polymer, although fluoropolymer stapes and ossicles are also used. Artificial devices (bionic hearing) utilize an external computer, where eight, tiny, polymer coated wires are implanted in the inner ear, connected to a nickel-sized plastic plug behind the ear. Silicone rubber can be used as an artificial eardrum membrane: PF and Teflon have been used for stapes prosthesis[187].

Many different materials have been tried in fabricating implants: PTFE, PE, silicone rubber, stainless steel, and tantalum. More recently, PTFE- carbon composite (proplast), porous PE (plastifore) and pyrolytic carbon (pyrolite) have been shown to

be suitable materials for cochlear (inner ear) implants[13].

Catheters for diagnosis of congenital heart disease, made of PVC or Teflon coated Dacron, contain additives that make them x-ray opaque. Tracheobronchial and urinary catheters are coated with PNVP[187]. Arteriography catheters, made of PU, are useful for diagnosing myocardial or cerebral infarction[187].

2.9 BIO-COMPATIBILITY ASSAYS

2.9.1 Introduction

For tissue compatibility minimum histotoxicity (nontoxicity), minimum tissue reaction and noncarcinogenicity are required. The toxicity test is described in the U.S.A. Pharmacopoeia. Most tests are intended to detect trace amounts of residues, modifiers, or additives. The cell-culture test is useful for *in vitro* analysis of biocompatibility. Incubation of cells on the test polymers gives information concerning the polymer-cell interactions, biocompatibility, and toxicity.

It should be recognized that different applications require different materials with specific properties. The following are minimal requirements for all soft tissue implant materials:

1- They should achieve a reasonably close approximation of physical properties, especially flexibility and texture.

2- They should not deteriorate or change properties after implantation with time.

3- They should not cause adverse tissue reaction.

4- They should be noncarcinogenic, nontoxic, nonallergenic, and nonimmunogenic.

5- They should be sterilizable.

6- They should be low cost[187].

The biological responses to polymer surfaces are exceedingly complex. They depend on the hydrophobicity or hydrophilicity of the surface; the smoothness, roughness, or porosity; the presence or absence of ionic groups; the types of elements exposed at the surface, bioerodability or biostability and whether the surface is that of a coherent solid or a hydrogel[186].

Blood compatibility is one of the most important required properties of biomaterials as thrombus formation, a combination of complex events, has to be rigorously excluded. Studies undertaken under the sponsorship of the NIH Artificial Heart program led to the development of a number of blood-compatible polymers.

2.9.2 Blood Compatibility Assays

Two important aspects of the interaction of foreign materials with blood are platelet adhesion and blood coagulation. These processes are dependent on surface properties of the material, on flow condition, and on the way in which the initial blood-material contact is brought about[191].

Hemocompatibility is one of the main requisites of materials used in the making of devices intended for contact with blood. Up to now there exists no biomaterial with a degree of hemocompatibility comparable to endothelium, which is the best non-

thrombogenic surface. All artificial surfaces activate platelets and coagulate plasma factor, although to different extents. Therefore, thrombosis of devices in contact with blood still represents a remarkably severe clinical problem[192].

One of the first observable events that occur as blood comes in contact (*ex vivo* or *in vivo*) with a polymer surface is platelet adhesion to that surface[193]. In order to characterize the interactions between constituents of the blood and the surfaces of polymers it is necessary to develop reproducible test methods which quantitatively describe the phenomena involved[189].

2.9.2.1 *In vitro* Test

2.9.2.1.1 Whole Blood Clotting Time

Albumin-coated vials were prepared by filling completely with an aqueous solution of 1% human albumin, a number of polystyrene vials of 0.5 cm^2 section area, previously washed thrice with anhydrous ethanol and desiccated. After incubation at 37 °C for 2 h, the vials were emptied, rinsed with a PBS solution, desiccated at room temperature and immediately used. Human venous blood, collected by clean venipunctures in plastic syringes without frothing, was divided into the albumin-coated vials each containing a film sample of 12 mm x 2 mm x 0.3-0.6 mm. The vials were gently tilted every 30 s until a clot was observed and this time was taken as the initial clotting time. For controls vials without any film sample were tested in the same way[133,194].

2.9.2.1.2 Platelet Rich Plasma (PRP) Method

Weighed amounts of polymers were taken in plastic disposable syringes (1 ml) and equilibrated with phosphate buffered saline (PBS) overnight. Buffer was squeezed out and freshly prepared PRP (0.3 ml) was introduced. The syringes were gently tapped to remove the air bubbles and incubated at 37 °C with continuous shaking to allow constant mixing of the PRP and glass beads. The syringes were taken out after the required time intervals, emptied into siliconized multilube tubes (1.5 ml) and aliquots were counted using a Coulter counter. Control samples prepared by incubating PRP without glass beads were used as references for each time point. Polymer-coated glass coverslips pre-incubated with saline for 1 h were incubated with PRP (freshly prepared) at 37 °C for 1 h, rinsed with saline and treated with 2.5 % glutaraldehyde in saline at 20 °C overnight. Following critical point drying and gold coating, the polymer surfaces were observed using a scanning electron microscope[194].

2.9.2.1.3 Quantitation of Adsorbed Fibrinogen

Polymers were allowed to adsorb on DDS-glass and LDPE tubings for 1 h at room temperature. Non-adsorbed polymers were removed from the surface by rinsing with at least 50 ml of PBS. Fibrinogen was adsorbed at the bulk concentration of 0.1 mg/ml for 1 h at room temperature on to the polymer surfaces. Excess fibrinogen was removed by rinsing with PBS. The surface fibrinogen concentration was determined by measuring the radioactivity of ^{125}I-labelled fibrinogen using a gamma counter. Eight samples from two independents were used for the calculation of the surface fibrinogen

concentrations[195].

2.9.2.1.4 *In vitro* Evaluation of Shunt Valve

A modified tube of 10 cm length was fixed in U-form within a glass tube of 35 mm bore. A glass capillary of U-form was also prepared as a control. Canine serum with 10 mM EDTA or 10 mM ethylene glycol tetraacetic acid (EGTA) and 5 mM Mg^{2+} was injected into the inside of the U-tube. After the serum was incubated in the specimen for 2 h at 39 °C, the remaining complement activity in the serum was assayed. Hemolytic activity of the whole complement and of the fourth component of complement were determined by the method of Mayer and that of Gasther and co-workers, respectively[175].

2.9.2.1.5 Lactate Dehydrogenase (LDH) Activity Method

Since platelet adhesion to a biomaterial surface is important, as it results in the formation of a haemostatic plug or thrombus, platelet number counting is one of the most popular experimental tools for evaluating the hemocompatibility properties of man-made materials.

To determine the number of adhered platelets, 2 ml lysis buffer (0.5% Triton X 100) in PBS was added to the films in a test tube. The lysis was allowed to proceed for at least 1 h at room temperature to ensure complete platelet disruption. The LDH activity of lysate was measured by addition of 0.3 ml substrate buffer to the tube. The change in ultraviolet absorption at 340 nm was measured immediately, using an ultraviolet

spectrometer. The initial linear part of curve was used for calculation of the LDH activity and the LDH calibration curve was obtained by measuring the enzymatic activity of a set of samples with a known concentration of platelets in PBS buffer under the same condition as the film[4].

2.9.2.2 *Ex Vivo Test*

2.9.2.2.1 *Ex Vivo* Arterio-Venous Shunt Experiment

Rabbits weighing 2-3 Kg were anaesthetized and their carotid artery and Jugular vein were exposed surgically to make an arterio-venous (A-V) shunt. The polymer tube was connected to the shunt circuit with the help of a vessel tip made of fluorine polymer. After removal of the tube from the shunt, the blood-contacting surface of the tube was rinsed with 100 ml PBS at 1.33×10^5 pa and fixed with glutaraldehyde for SEM[135,146].

2.9.2.3 *In Vivo* Test

2.9.2.3.1 *In vivo* Evaluation of Shunt Valve

The modified tubes were implanted in the external jugular and femoral veins of mongrel dogs. In order to correct differences among individual dogs, an original PE tube was always implanted in each dog as a control and the relative occlusion time for

the modified tubes was given by standardization with the occlusion time of the control. To study the role of complement in thrombus formation, dogs were decomplemented by the intravenous injection of CVF(Naja Haje; 60-70 U/Kg body wt) 20 h before implantation. According to a patent the occlusion time, by thrombogenesis, was measured by use of an ultrasonic blood-flow metre. Some specimens were also excised 30-60 min after implantation to observe cell adhesion on the luminal surfaces[175].

2.9.2.3.2 Vena Cava Ring Test

Rings (length= 9 mm, O.d= 8 mm, I.d= 7 mm) with stream-lined leading and trailing edges (to prevent turbulence), fabricated from the test material and / or coated onto substrate materials, were implanted in the inferior vena cava of the dog. Candidate materials were initially subjected to a 2-h ring implant test. If the materials showed promising thromboresistance, then additional rings were implanted for 2 weeks (chronic test) with no animal used more than once. The rings were inserted with the aid of a special device to ensure that they do not touch the atrial wall and become contaminated with tissue fluid as the rings pass into the vena cava. The test rings were sterilized either by the materials developer or by the testing group according to the recommendations of the developer[169].

2.9.3 Tissue Compatibility Assays

The body has three basic responses to the implantation of a foreign body. First, it responds to the physical characteristics of the object (shape, roughness, presence of

sharp edges, *etc.*). These responses may take the form of epithelial encapsulation of the foreign body, keratinization of the surrounding tissue, thickening of the connective tissue, or generation of giant cells. Second, the body reacts to the chemical toxicity of the polymer by the appearance of tissue inflammation, inhibition of epithelial growth, and other effects. Finally, there is a possibility of bacterial, fungal, or viral infection originating at the surface of the implant or of the polymer surface[186].

Any program of assessment on biomaterials must include biocompatibility tests on cell cultures as stated by the main standards agencies[196].

2.9.3.1 *In Vitro* Test

Cell culture systems may be of value in testing the biocompatibility of prosthetic materials before they are introduced into clinical use. In recent years, *in vitro* methods for assaying biomaterials have gained in importance owing to the growing concern over the use of animals for biomaterials testing, significant effort is therefore being focused toward developing predictive and quantitative but also simple and reliable, methods of testing using cultured cells. At present, a number of methods for measuring both the cytotoxicity and the specific cytocompatibility of different materials are available. The usefulness of these systems is no longer confined to screening new materials, they can be used to study the mechanisms of action of various materials during tissue material interaction[196].

2.9.3.1.1 Cell Culture Method

Polymers were placed in individual wells of a 24-well multiwell dish and sterilized by UV radiation. Human normal umbilical cord fibroblast cells, which were maintained in Dulbecco's modified Eagle's medium supplemented with 5% fetal calf serum, streptomycin (3.0×10^{-3} wt %) and penicillin (5.0×10^{-3} wt%) were harvested with trypsin (0.15%, 2000 units/g), 0.02% EDTA, (-) PBS (PH 7.4) and resuspended in DMEM at a concentration of 1×10^5 cells /ml. The cell suspension (1 ml) was seeded on to the polymer placed in a multiwell dish and incubated at 37 °C in an atmosphere of 5 vol% CO_2. After the required incubation period, cells were washed three times with PBS with gentle shaking of the dish and detached using 0.2% EDTA - 0.15% trypsin - PBS by incubating at 37 °C for 2 h. Complete detachment of cells was ensured by an optical microscope. Cell numbers in suspension were counted using a Coulter counter[194,197].

2.9.3.1.2 Cell Adhesion

Assay to measure the strength of cell adhesion to surfaces are useful in characterizing cell interactions with biomaterials. One common approach is to expose adherent cell to steady laminar flow and measure cell detachment as a function of the wall shear stress. Cell attachment assays were used to study the effect of the surface composition and structure of the substrate materials on soft tissue response. The cells that could aid in gingival attachment are epithelial and fibroblast cells; therefore, these cells were chosen for the assays[198].

2.9.3.2 *In Vivo* Test

The *in vivo* tests were performed using two specimens of 1 cm^2 for any chosen film sample. The specimens were implanted intraperitoneously in adult male Wistar rats, weighing 200-250 g and fed *ad libitum*, and the wounds were closed by stainless steel clips. For control, two rats were operated on with the same procedure but no material was implanted. All the animals were killed after 25 days[133].

CHAPTER 3

EXPERIMENTAL SECTION

3.1 MATERIALS

3.1.1 PDMS Vulcanization

The substrate polymer used for pre-irradiated CO_2-pulsed laser and then deposition polymerization was polydimethylsiloxane, M3090 medical grade which was purchased from Waker. Polydimethylsiloxane rubber (PDMS) is usually vulcanized by heating with organic peroxides. Raw polydimethylsiloxane rubber was masticated in a two roll mill external mixer (LAB, two roll mill polymix, 200L, type U) at 80°C for 5 minutes. The warm rubber was milled with 1 phr dicumylperoxide (DCP, 99%) at 80°C for 5 minute. To find out the suitable DCP concentration and optimized cure condition, raw PDMS was mixed with different DCP levels and their curing characteristic was evaluated using a rheometer. Finally, 0.8 phr of DCP was found to give a strong and elastic network at 165°C and 5 minute of curing. The rubber was formed into 2.0 mm thick sheets and compression cured at 165 °C for 5 min. Vulcanized films of 0.3 mm thickness and 3.5 × 3.5 cm dimensions were Soxhlet extracted with toluene : methanol (60:40 v/v) for 24 h and then dried in a vacuum oven at ambient temperature to constant weight.

Figure 3.1 shows the ATR-FTIR PDMS rubber vulcanized. It can be seen that PDMS has significant absorption within 9-11 μm (909-1111 cm^{-1}). We have chosen PDMS due to it possessing some excellent properties, such as high structural resistance towards

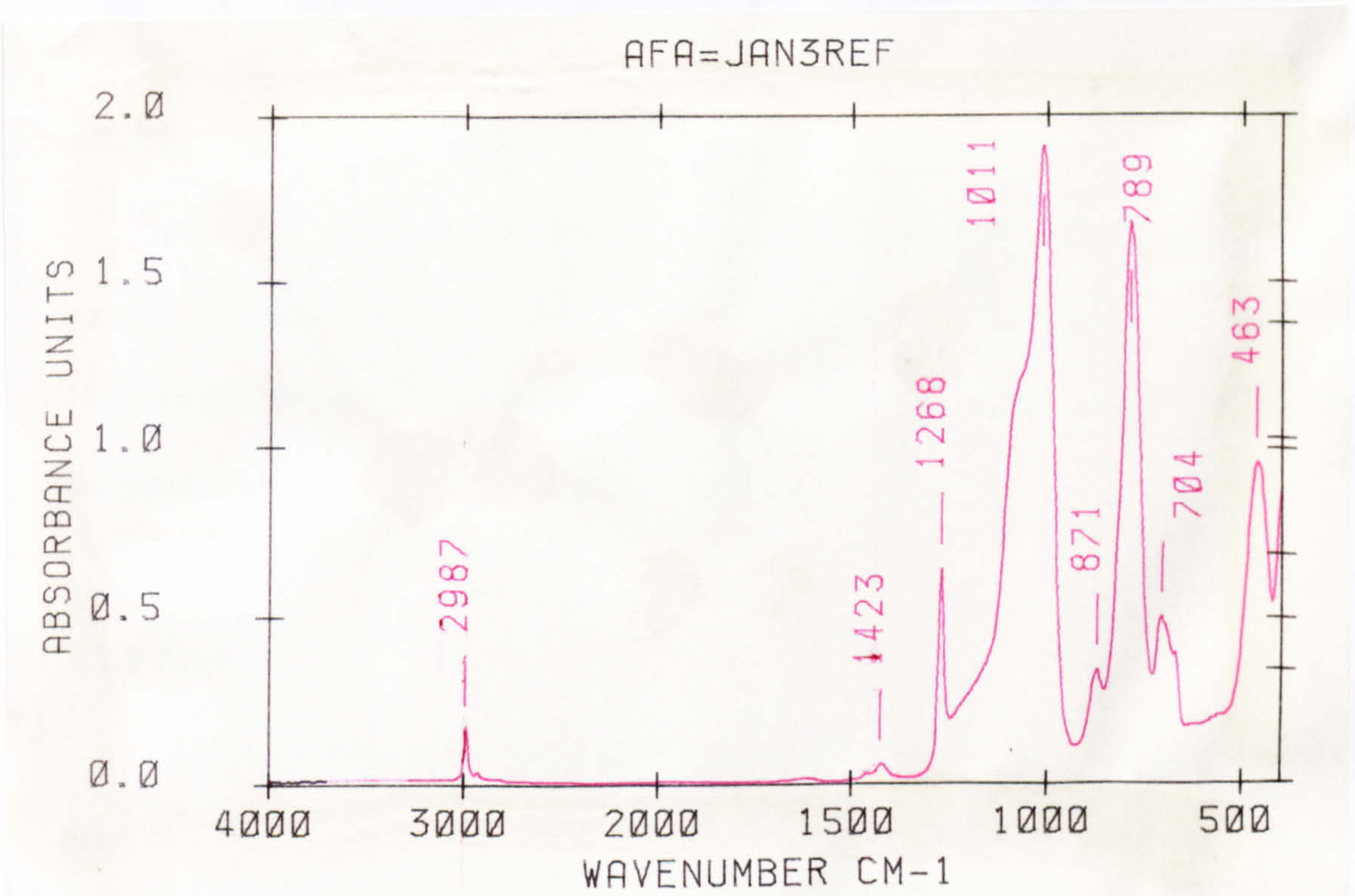


Figure 3.1 ATR - FTIR spectra of polydimethylsiloxane.

bio-invasion, heat, ozone and chemicals, very good bulk properties, inertness, nontoxicity and that it can readily be processed to form complex shapes and sizes according to their final application.

3.1.2 Acrylamide

Acrylamide (AAm > 99 %) from Aldrich was recrystallized from chloroform

prior to use.

3.1.3 HEMA and HEMAPC

HEMA (>99%) from Aldrich was purified through an alumina column and by passing distillation at 42°C (1 mmHg) respectively prior to use. 2-Methacryloyloxyethyl- 2'-(trimethylammoniumethyl) phosphate, inner salt from Biocompatibles Ltd (UK) was used as received.

3.1.4 Eosin

The occurrence of grafting and change on the surface of the rubber and for platelet adhesion study were followed using staining with eosin which was used as received from Aldrich. (see the procedure of staining in section 3.6)

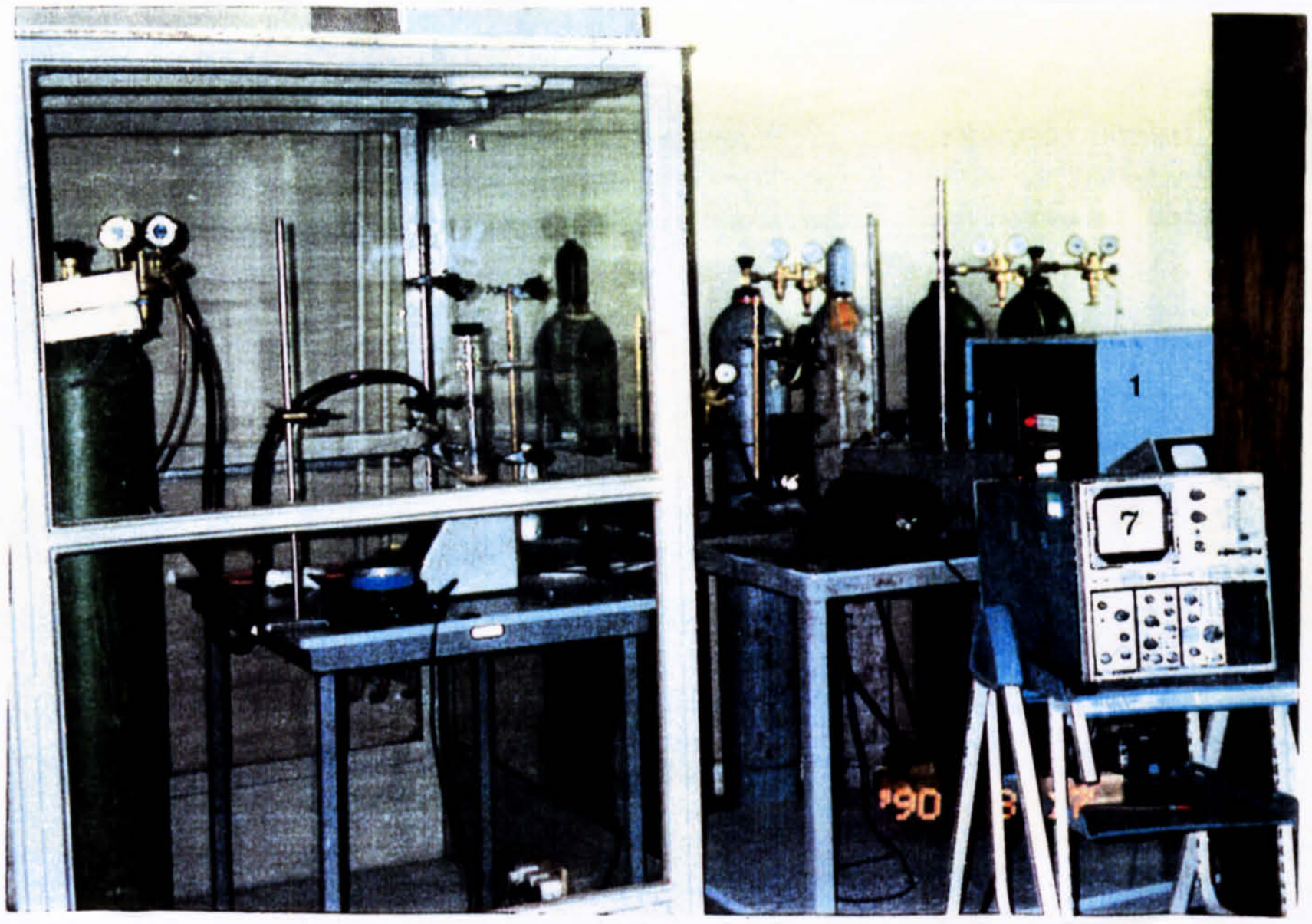
3.2 Irradiation Procedure

CO_2-pulsed laser-induced surface modification of PDMS and laser graft copolymerization of AAm, HEMA and HEMAPC onto PDMS was performed by the simultaneous and continuous technique. For this purpose a step-motor equipped with a belt was made so that the laser beam could be directed vertically onto the surface of PDMS samples which were laid on the belt. Both sides of the sample surfaces were treated and the whole surface was scanned by the laser pulses in oxygen, nitrogen and air conditions and neither chemical nor photosensitizer were used. The laser used was

a line-tunable pulsed CO_2-laser (TEA CO_2 laser Lumonics-103-2) which provides laser beams of wavelengths from 9.1 - 10.6 μm (1098 cm^{-1} - 943 cm^{-1}) and a pulse fluence between 0.8-5 J/cm^{-2}. The experimental apparatus and CO_2-pulsed laser diagram are shown in Figures 3.2. The pulse duration was 100 nano second (ns). The uniformity and the full width at half maximum (FWHM) of the pulses was checked by burn patterns obtained on thermal paper at different fluences and recorded using a Tektronics oscilloscope (7844) operating at 500 MHz. Each sample was exposed to the laser pulses under the selected conditions [laser beam diameter 3.5 × 3.5 cm (obtained through use of a beam expander), fluency 1-5 J/cm^2, wavelengths 9.1- 10.6 μm (1098 cm^{-1} - 943 cm^{-1}) and repetition rates 0.1 - 1.5 Hz]. After each exposure, the samples were removed from the belt of the step-motor and washed first with acetone and then thoroughly extracted with acetone: distilled water (50:50 v/v) at 80°C for 48 h. The extracted sample was dried in a vacuum oven at 50°C to constant weight.

3.3 Surface Characterization and Analysis

In order to characterize the surface of the modified samples the following methods were used.



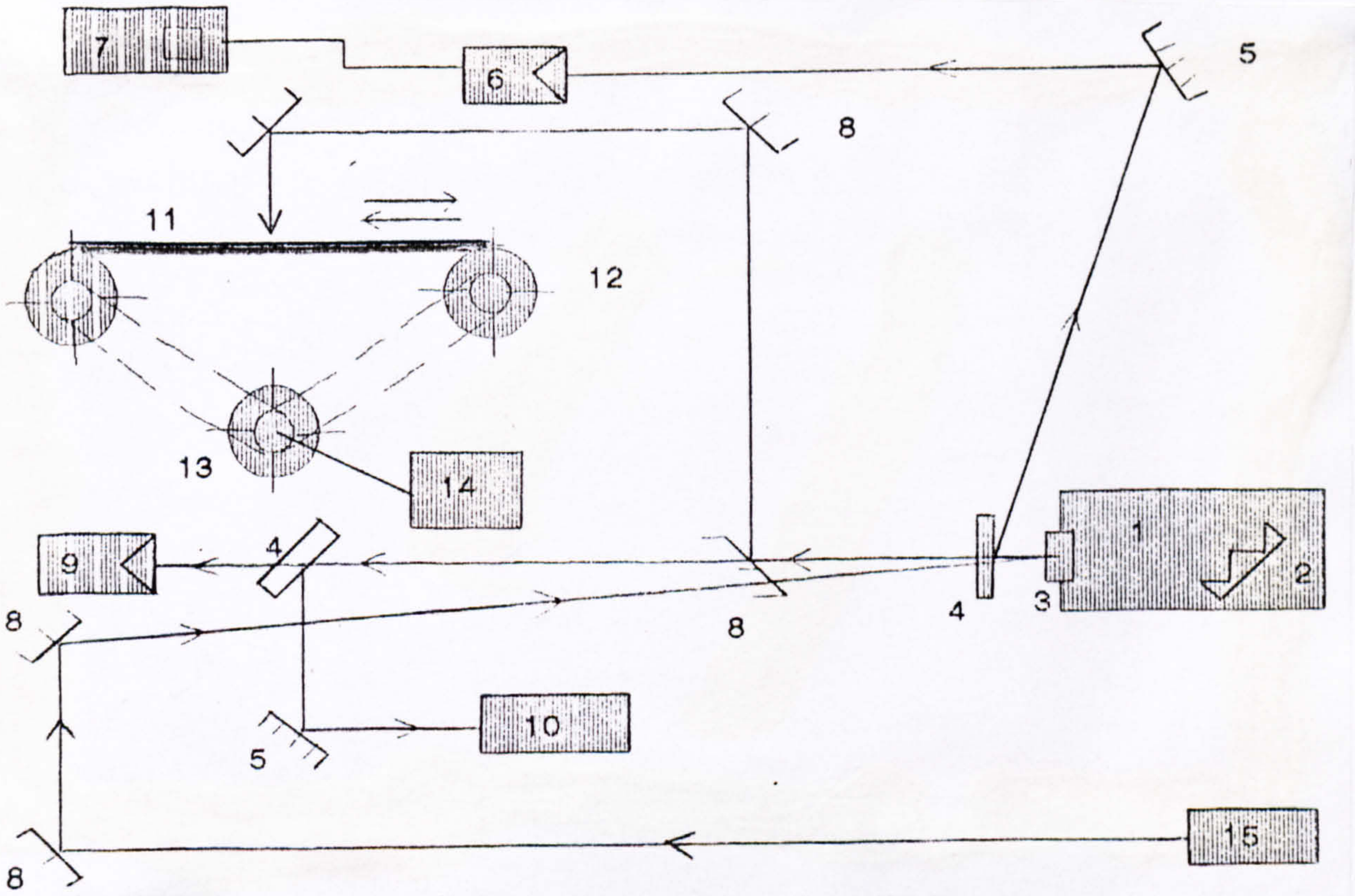


Figure 3.3 **Diagram of the used CO_2-pulsed laser radiation system. 1- TEA CO_2-pulsed laser; 2- Grating 150 L/mm; 3- Ge-mirror; 4- NaCl crystal; 5- Concave 100% reflector mirror r = 20 cm; 6- Photon array detector; 7- Tectronics oscilloscope (7844), 500 MHz; 8- 100% reflector mirror; 9- Scintech Inc. power metre; 10- Optical engineering Inc. CO_2 spectrum analyser; 11- Belt of PDMS; 12- roller; 13- Screen; 14- Step motor; 15- He . Ne laser.**

3.3.1 ATR-FTIR

In order to characterize extracted, laser treated PDMS and AAm, HEMA, HEMAPC grafted onto PDMS samples, attenuated total reflectance ATR-FTIR with KRS-5 prism and an incident angle of 45° was used. The absorption bands at 1011 cm^{-1} attributed to the -Si-O-Si- group of the polydimethylsiloxane were measured. A Brucker - 88 FT-IR spectrometer (Figure 4.3) with adequate nitrogen purging was employed using spectra generated from 50 scans.

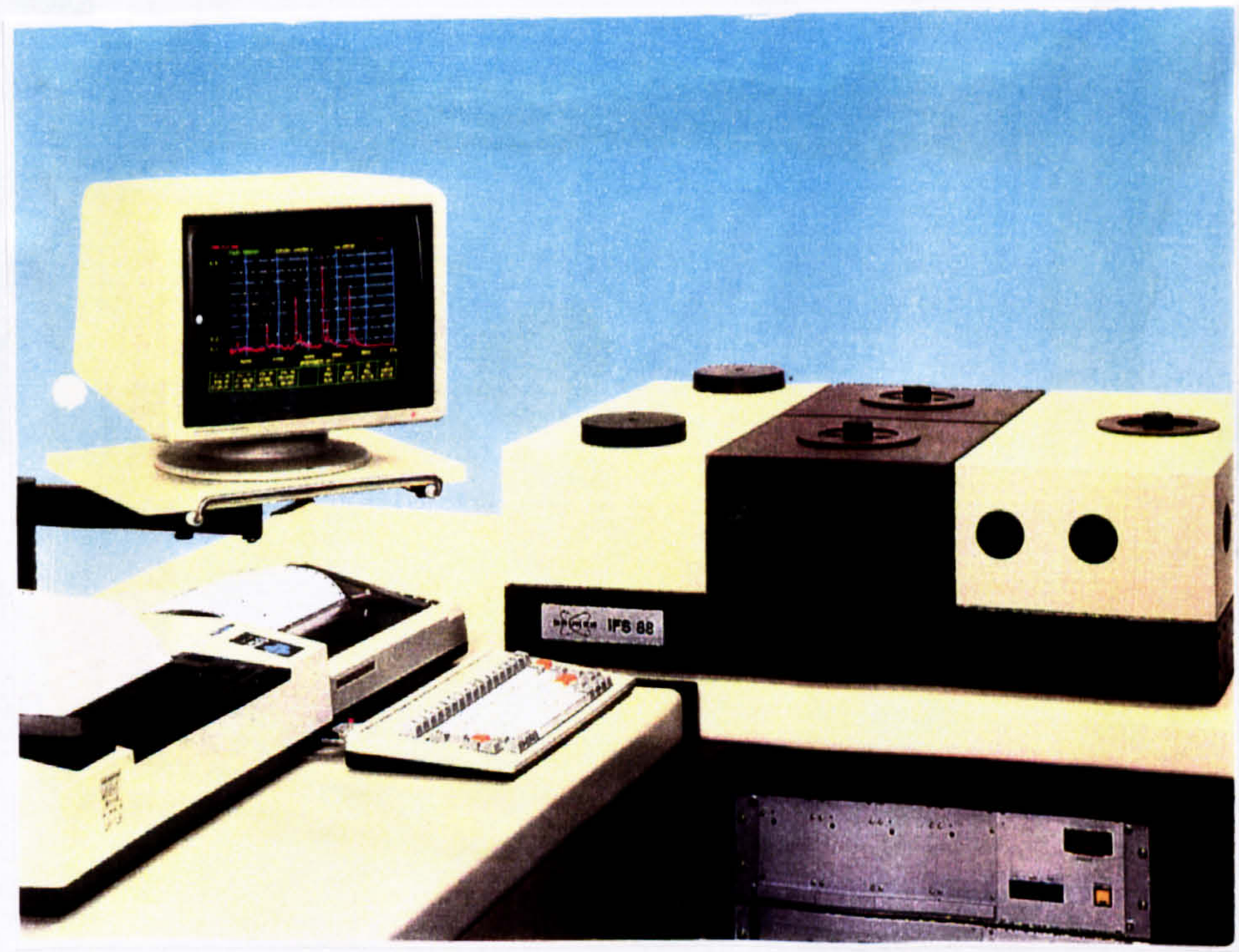


Figure 4.3 Brucker - 88 FT-IR spectrometer used.

3.3.2 SEM

Scanning electron microscopy (SEM) was performed on gold-coated samples using a Polaron sputter coater. A Cambridge S-360 SEM (Figure 3.4) operating typically at 10, 5 kV and tilt 0° was employed for the morphology measurement and evaluation of homogeneity of the modified samples.

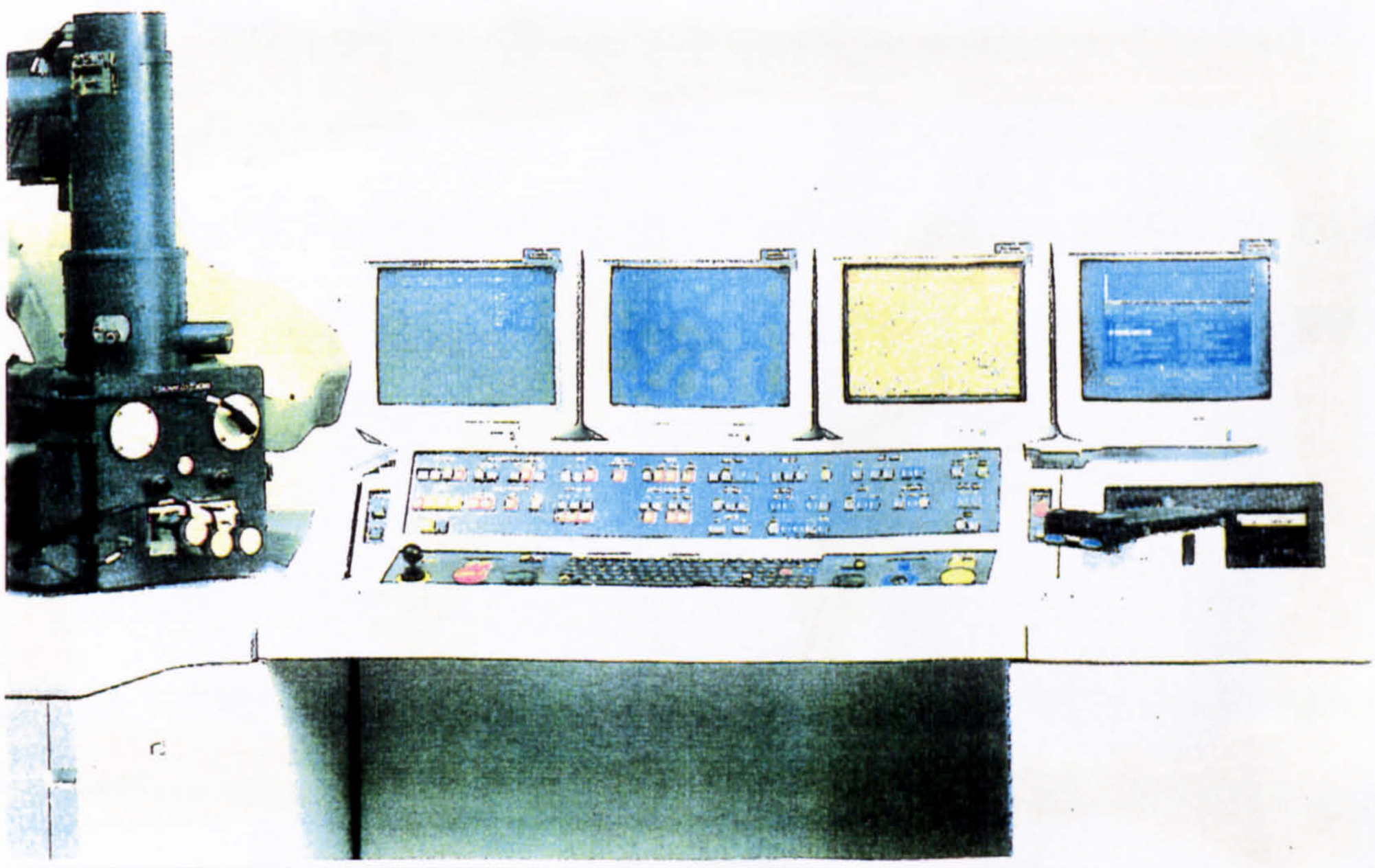


Figure 3.4 Cambridge S - 360 SEM used.

3.3.3 EDXA

Energy dispersive X-ray analysis was used to measure the depth and homogeneity and O/Si ratio and acrylate grafting of the modified samples. A thin beryllium window and an AN-10000 EDXA unit, coupled to the SEM, were employed. Voltages of 5-6 kV were necessary to give appropriate Kα lines of C , O , Si elements of PDMS.

3.4 Dynamic Mechanical Thermal Analysis (DMTA)

The dynamic mechanical properties of the modified and unmodified samples were studied and compared using a model PL DMTA analyser (Polymer Laboratories DMTA). The samples were vibrated in bending mode (single cantilever) at 0.1, 1, 10 Hz and at temperature from -100 to 100 °C. For each sample, the storage modulus (É) and the loss tangent (tan δ) versus temperature were recorded.

3.5 Contact Angle Measurement (Wettability)

Water drop contact angle was evaluated by measuring the contact angle formed between water drops and the surface of the modified samples. For this purpose, the drops of water were mounted on five different areas of the surface with a microsyringe for 5 min then photographs were taken at 50 x . All contact angles are the means of five determinations.

3.6 Staining

The occurrence of grafting and change on the surface of the rubber and showing the platelet adhesion on the different samples of PDMS, staining by eosin were used. Small pieces (typical sample cross-sections being about 0.3-1 mm thick), were immersed in either 2.5% eosin in a 3:1 water methanol solution for 3 h. After staining, samples were washed in distilled water for two h to remove excess stain, and then dried prior to microscopic examination. Coloured samples were observed in a light microscope. Polyacrylate grafted PDMS was stained with eosin for 3 h at room temperature. The stained PDMS films were sectioned with a microtome and put on a slide glass and grafted layer was observed with an optical microscope (Zeiss).

3.7 Friction Coefficient measurment

To quantitatively characterize the surface slipperiness the friction coefficients of the untreated and modified PDMS surfaces were measured in the dry state according to the ASTM D 1894-78 method by using Friction Measuring Apparatus (Davenport). Both static and dynamic methods were used.

3.8 Graft Polymerization of Acrylamide

Graft polymerization of AAm, HEMA, HEMAPC onto the PDMS surfaces were performed according to the scheme briefly illustrated in Figure 3.5 After laser treatment peroxides on the surface were introduced. Graft polymerization of acrylate

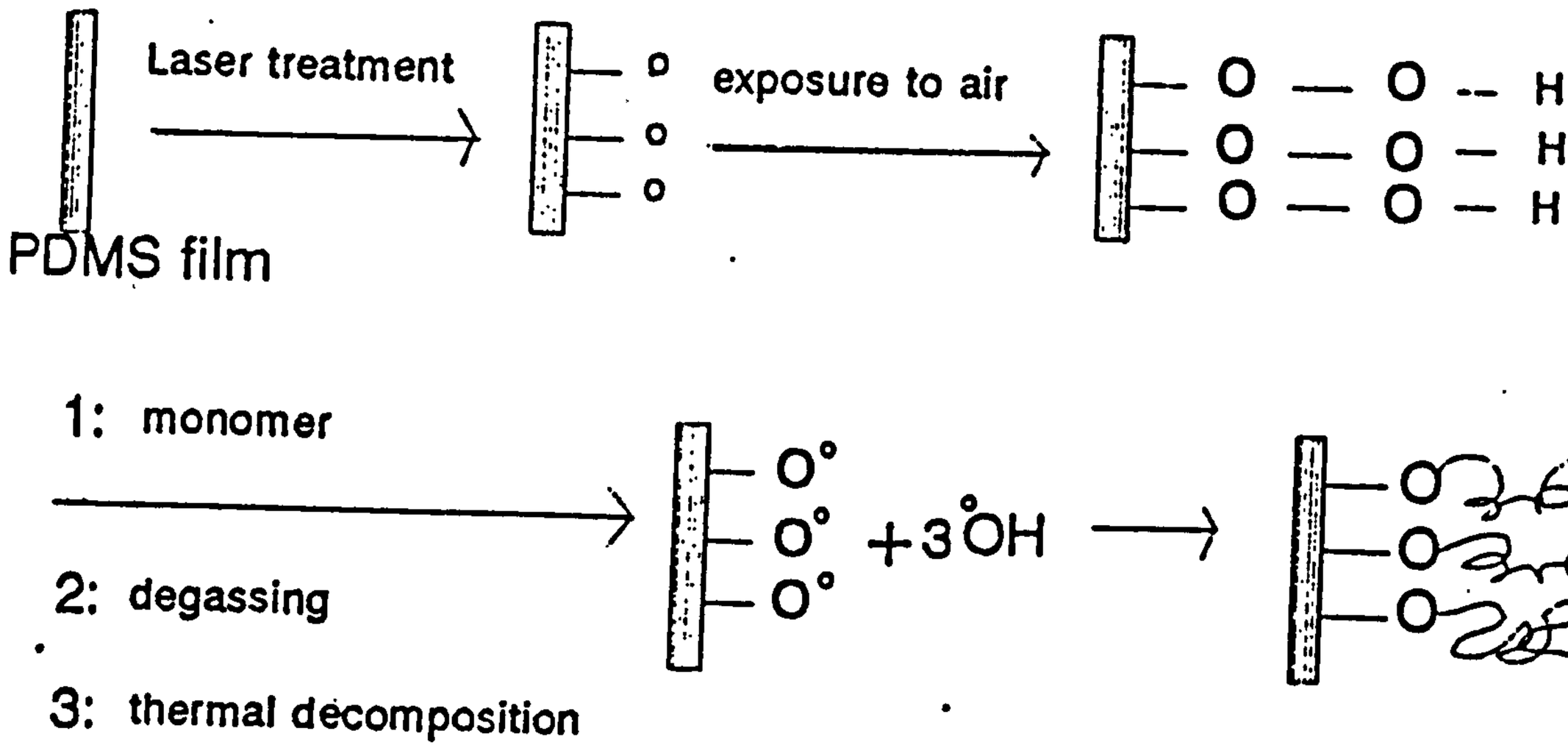


Figure 3.5 **Schematic illustration of the CO_2-pulsed laser graft polymerization of AAm, HEMA, HEMAPC onto the PDMS pre-treated in oxygen atmosphere with CO_2-pulsed laser.**

monomers onto the treated silicone were carried out using the apparatus illustrated in Figure 3.6 Two separate reactors which can be connected to each other were used, one for degassing of the CO_2 laser treated PDMS, and the other for deaeration of the acrylate solution. Between them there is a valve which alters the introduction of

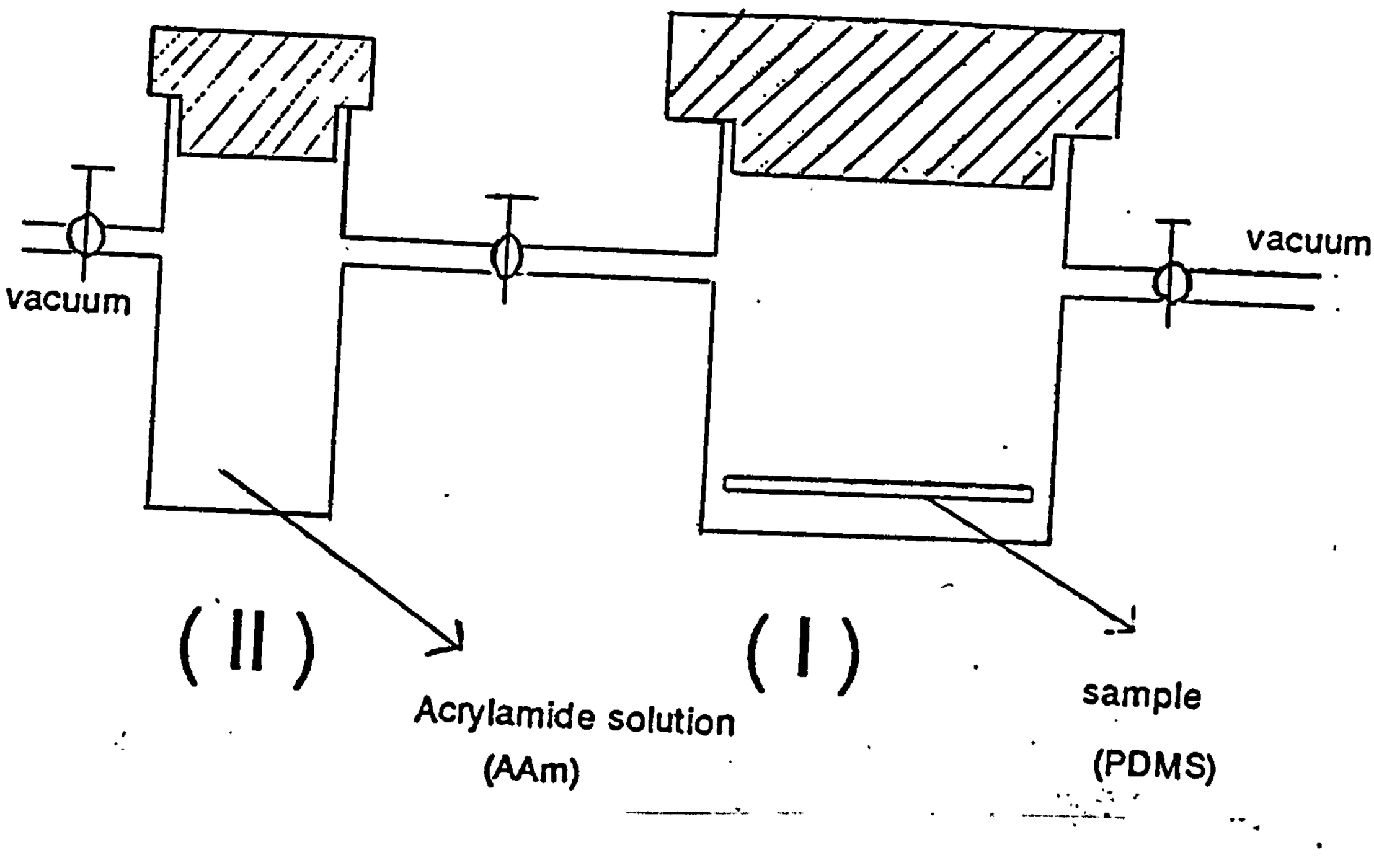


Figure 3.6 Reactors used for graft polymerization of AAm, HEMA, HEMAPC onto the PDMS pre-treated with CO_2-pulsed laser.

acrylate solution into the PDMS sample reactor. After the laser treatment for a specified pulse number, the treated PDMS is placed in part I. Following degassing with a vacuum pump for 6 h under a pressure of 0.2 mm Hg, the valve is closed, then separately 1, 5, 10, 20, 30, wt % acrylate aqueous solutions (depending on acrylate) is poured into part II, and the degassing was carried out by freezing the solution with N_2 liquid. After three times degassing, the solution was poured into part I without

exposing to air. Graft polymerization of acrylate onto the treated PDMS is allowed to proceed at 50 °C for 2 h. After removal of acrylate homopolymer with water at 80 °C for 24 h, the increased weights were determined.

3.9 Peroxide Determination

Polymeric peroxides introduced on the treated films were determined by the following two methods:

a- A flask containing the irradiated film and 20 ml isopropyl alcohol, to which 5 ml isopropyl alcohol solution saturated with sodium iodide and 2 ml glacial acetic acid was added, and refluxed at 85°C for 15 min. After being cooled to room temperature, the liberated iodine was titrated with 0.01 N sodium thiosulphate solution[199].

b- The treated films are put in a benzene solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH, radical scavenger) and kept at 70 °C for 24 h to decompose the peroxides. The DPPH molecules consumed by binding to the radicals formed are quantified from the difference in transmittance at 520 nm between the virgin and the treated film using 1.18 x 10^4 L mol^{-1} cm^{-1} as the molar absorptivity of DPPH[199,200].

3.10 *In Vitro* Tests

3.10.1 Platelet Rich Plasma (PRP) Method

Venous blood from a healthy human was collected with a vacuum syringe containing 5% citric acid. The blood was centrifuged at 800 rev min for 10 min at 25 °C and the platelet rich plasma (PRP) was withdrawn with a PE pipette and placed

in clean vials. The residue of the blood was centrifuged at 3000 rev min^{-1} for 10 min to obtain platelet poor plasma (PPP). The platelet count pf PRP was determined with Coulter counter (type 4) and adjusted to 150000 platelets mm^{-3}.

PRP (1 ml) was placed on each of the PDMS films of 1 cm^2 and allowed to stand for 1 h at 37°C. The samples were then vigorously washed with PBS and treated with 2.5 % glutaraldehyde in saline at 20°C overnight. The samples were then dehydrated with serial dilution of ethanol (50-100%) and dried up to critical point. The specimens were then sputter coated with gold and examined with SEM using 10 kv of accelerating voltage.

Platelets which were adherent to the surface were fixed with 2% (v/v) glutaraldehyde in PBS for 1 h and stained with 2.5% Eosin for 3 h at room temperature. The stained platelets were observed with light microscopy (Zeiss) at a magnification of 1000 X.

3.10.2 LDH method

Since platelet adhesion to a biomaterial surface is important, as it results in the formation of a haemostatic plug or thrombus, platelet number counting is one of the most popular experimental tools for evaluating the hemocompatibility properties of man-made materials. To determine the number of adhered platelets, PRP (1 ml) was placed on a polymer film of 15 mm diameter in a 24 well multidish made of polystyrene and kept for 30 min or 1 h at 37°C under a static condition. The film was taken out and dip-rinsed twice with PBS(-). After washing, the film was put into a test tube. To determine the number of adhered platelets, 2 ml lysis buffer (0.5% Triton x 100 in PBS)

was added to the films in a test tube. The lysis was allowed to proceed for at least 1 h at room temperature to ensure complete platelet disruption. The LDH activity of lysate was measured by addition of 0.3 ml substrate buffer to the tube. The change in ultraviolet absorption at 340 nm was measured immediately using an ultraviolet spectrometer with a recorder. The initial linear part of curve was used for calculation of the LDH activity and the LDH calibration curve was obtained by measuring the enzymatic activity of a set of samples with a known concentration of platelets in PBS buffer under the same conditions as the film[4].

3.10.3 Cell Attachment

The Baby Hamster Kidney cells (BHK) were used as a model cell line in order to investigate the effects of cell morphologies on the cell attachment. BHK cells, which have fibroblast-like morphology, grow only in a monolayer culture. In the cell culture studies, the culture medium was Dulbecco's modification of Eagle's MEM for BHK cells. This media were modified by using 10% fetal calf serum and 100 μg/ml gentamicin. PDMS control and treated films sterilized by autoclave and swollen in PBS were placed into multiwell PS petri dishes and cell culture studies were performed in static culture conditions. The inoculation density was 1.78×10^5 cell/ ml. The attached cells on the PDMS films were determined at selected times. The cells adhering to and spreading on the surfaces were sputter coated with gold and examined with SEM using 10 kv of accelerating voltage.

BHK cells which were adherent to the surface were fixed with 2% (v/v) glutaraldehyde

in PBS for 1 h and stained with 2.5% Eosin for 3 h at room temperature. The morphology of the stained cells adhering to the substrate was observed by light microscopy (Zeiss) at magnification of 200 X. The amount of adhering cells per 1 mm^3 was determined by counting the number of cells after detachment in culture medium of the substrate surface.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Laser Treatment

The surface modification of sheets of polydimethylsiloxane (PDMS) by use of CO_2-pulsed laser as the excitation source, without photosensitizers, was studied at room temperature. The modified surfaces were characterized using a variety of techniques including scanning electron microscopy (SEM) combined with energy dispersive X-ray analysis (EDXA), attenuated total reflectance infrared (ATR-IR) spectroscopy, friction coefficient and the water drop contact angle analysis.

4.1.1 ATR-FTIR Study of the Laser Treated PDMS in Different Atmospheres

Figure 4.1(a-d) shows the ATR-FTIR spectra of the PDMS samples treated with 1, 5, 10 and 15 pulses of the CO_2 laser in an oxygen atmosphere at the wavelength of 9.58 μm (1043 cm^{-1}) which corresponds to the maximum absorption bond of -O-Si- of PDMS and is compared with the unmodified material (Figure 4.1e). As was discussed in section (2.2) due to the high coherency and monochromaticity, laser induced photochemical reactions such as oxidation can occur providing the laser is tuned to the absorption bonds of one the species in the system. The intensity of this group (-Si-O-) was reduced with increasing numbers of the laser pulses, in comparison with the

spectrum of the untreated sample. As shown in Figure 4.1(a-d) the absorption bonds at 789 cm^{-1}, and 1268 cm^{-1} were also decreased, representing the valence vibrations of the -Si-C- and -C-H groups respectively.

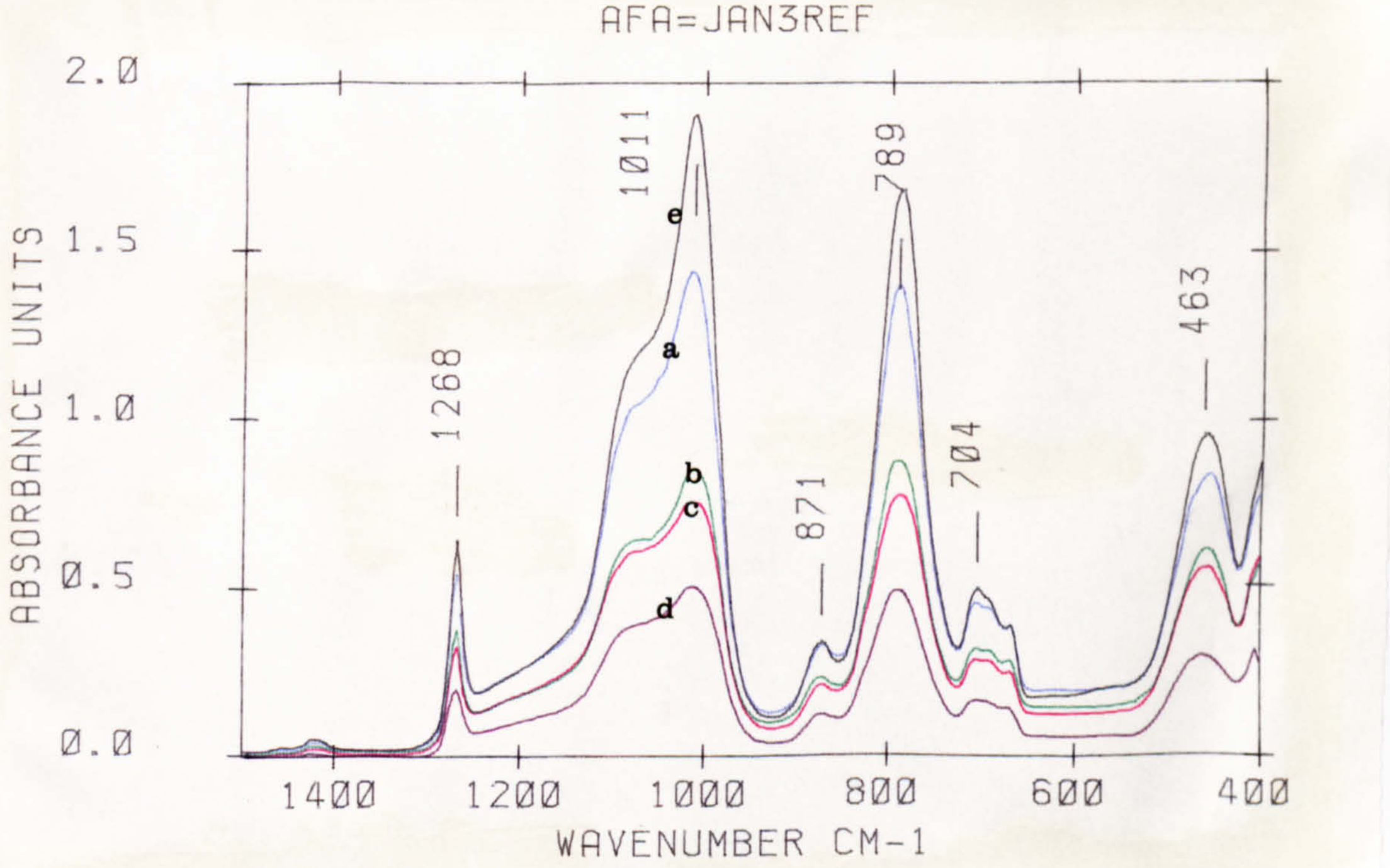


Figure 4.1 ATR - FTIR spectra of the PDMS treated with the CO_2-pulsed laser at oxygen atmosphere at wavelength of 9.58 μm (1043 cm^{-1}): sample (a) 1 pulse treated; (b) 5 pulses treated; (c) 10 pulses treated; (d) 15 pulses treated; (e) untreated PDMS.

These reductions in the intensity of the 1011 cm^{-1} bond and also in other bands are an obvious manifestation of the intense fragmentation on the surface of PDMS. Figure 4.2a indicates the modified PDMS exhibited a strong absorption at 1746 cm^{-1} which was assigned to carbonate groups (-O-COO-) on the surface of laser irradiated PDMS by 5 pulses compared to the unmodified sample (Figure 4.2b).

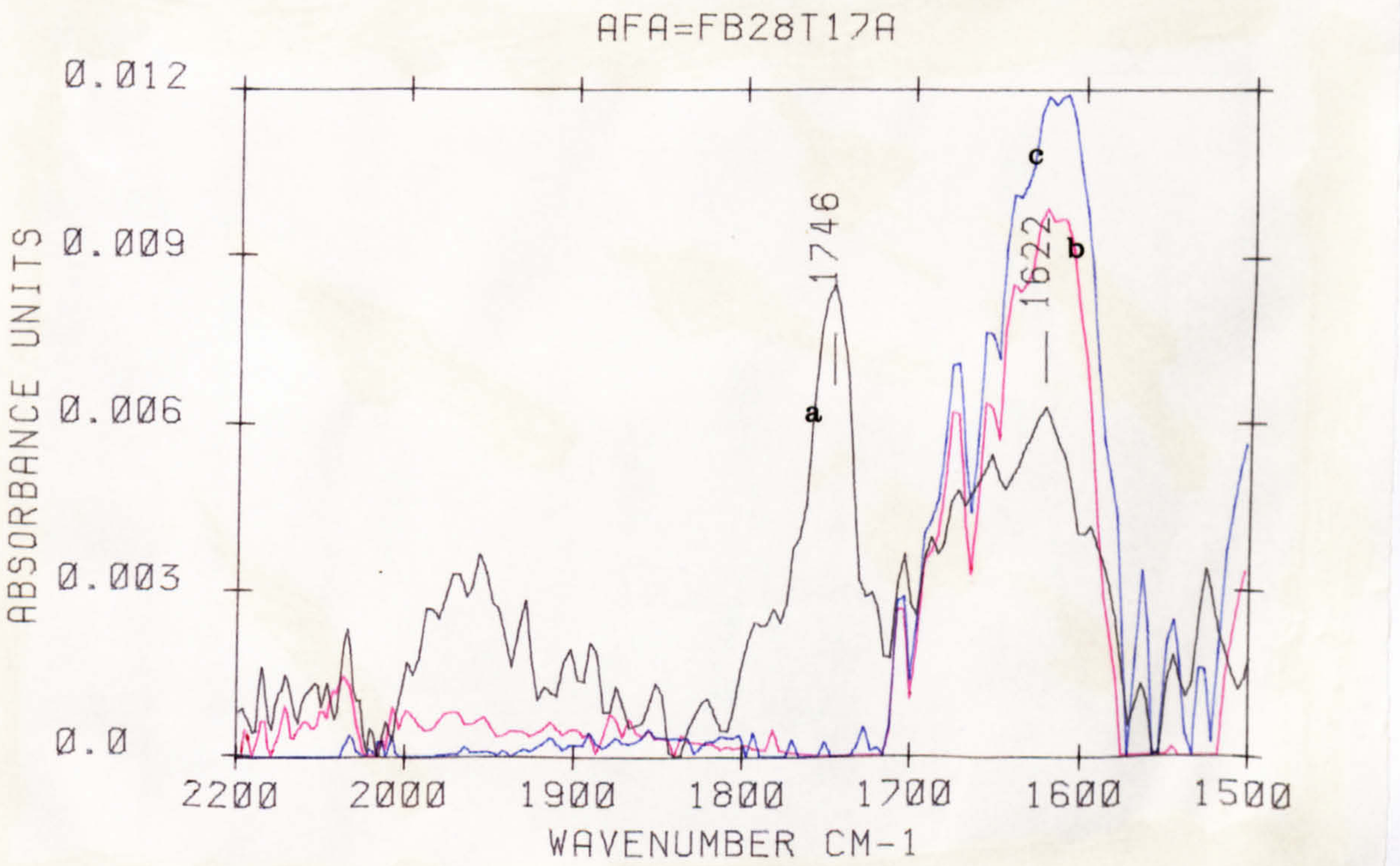


Figure 4.2 ATR - FTIR spectra of PDMS, (a) treated with the CO_2-pulsed laser by 5 pulses at oxygen atmosphere at the wavelength of 9.58 μm (1043 cm^{-1}); (b) untreated PDMS; (c) as in (a) but under nitrogen atmosphere.

This intense peak (1746 cm^{-1}) has not been observed in an oxygen-free atmosphere (Figure 4.2c, nitrogen atmosphere). This peak give an evidence of oxidation reactions as a result of the laser irradiation at 9.58 μm (1043 cm^{-1}) wavelength. It was observed that the induced changes were reproducible. Figure 4.3 shows the ATR-FTIR spectra of the laser treated PDMS versus pulse number between 1500-2000 cm^{-1}.

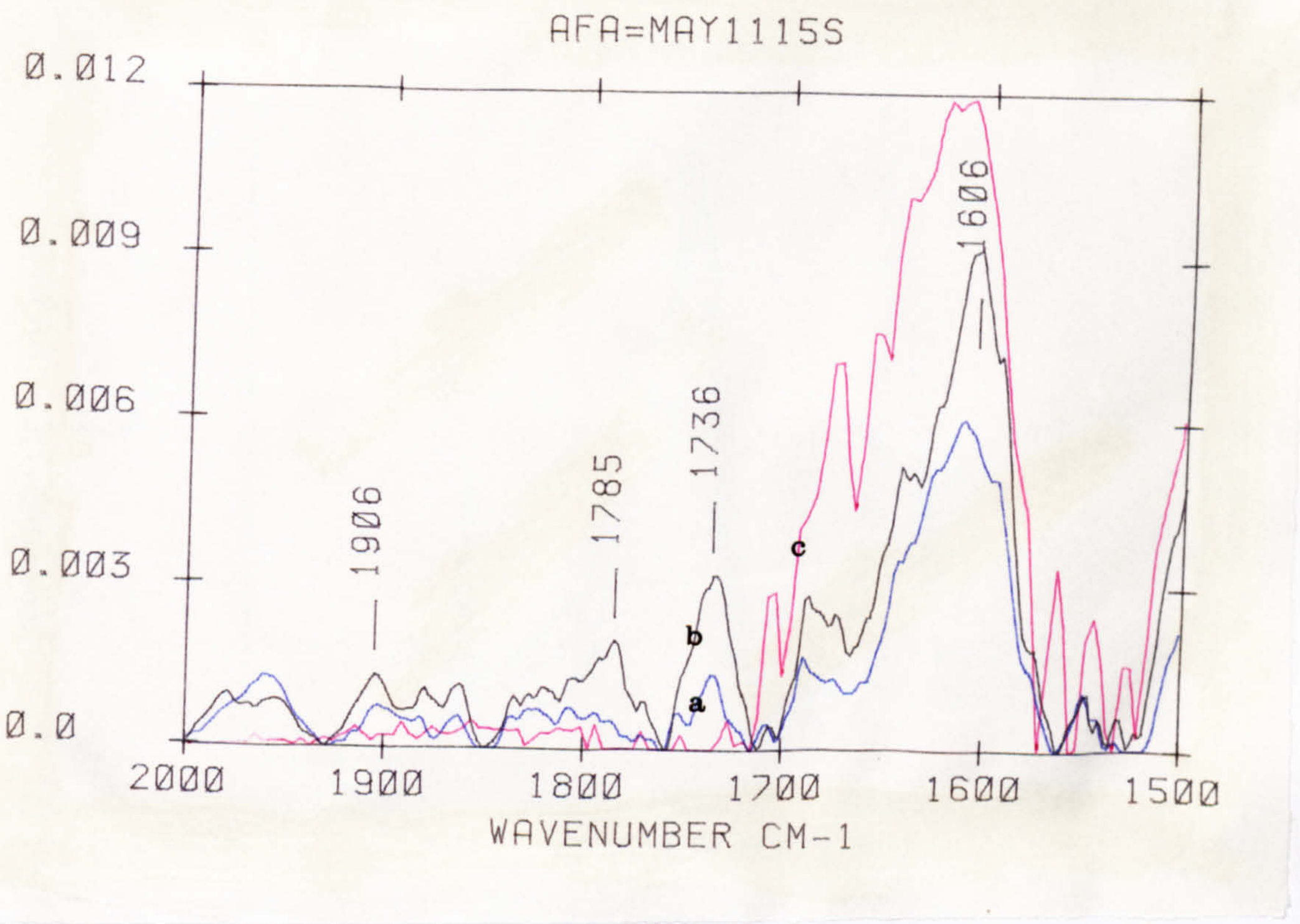


Figure 4.3 ATR - FTIR spectra of PDMS: (a) treated with the CO_2-pulsed laser by 1 pulse at oxygen atmosphere at the wavelength of 9.58 μm (1043 cm^{-1}); (b) treated with the CO_2-pulsed laser by 2 pulses at the wavelength of 9.58 μm (1043 cm^{-1}); (c) untreated PDMS.

As can be seen in this figure, by increasing the laser pulses, the intensity absorbance of this group (1746 cm^{-1}) also increases which indicates that the oxidation reaction process is increased by increasing the number of laser pulses. Figure 4.4 shows the ATR-FTIR spectra of laser treated PDMS in a nitrogen atmosphere. The trend of decreasing of intensity of the -Si-O- and -Si-C- and C-H groups is the same as shown for oxygen treated PDMS but in an oxygen atmosphere , the decreasing of intensity

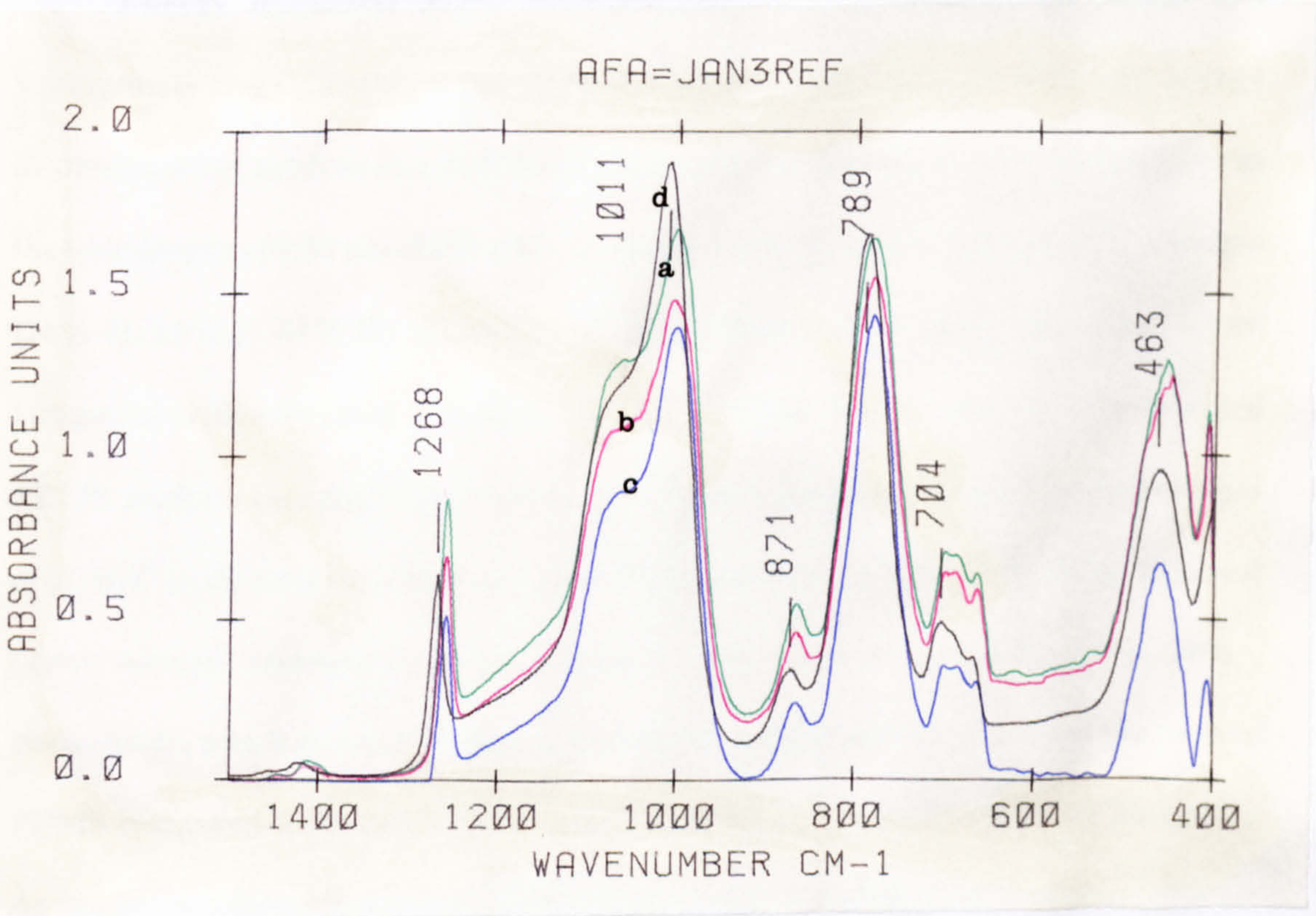
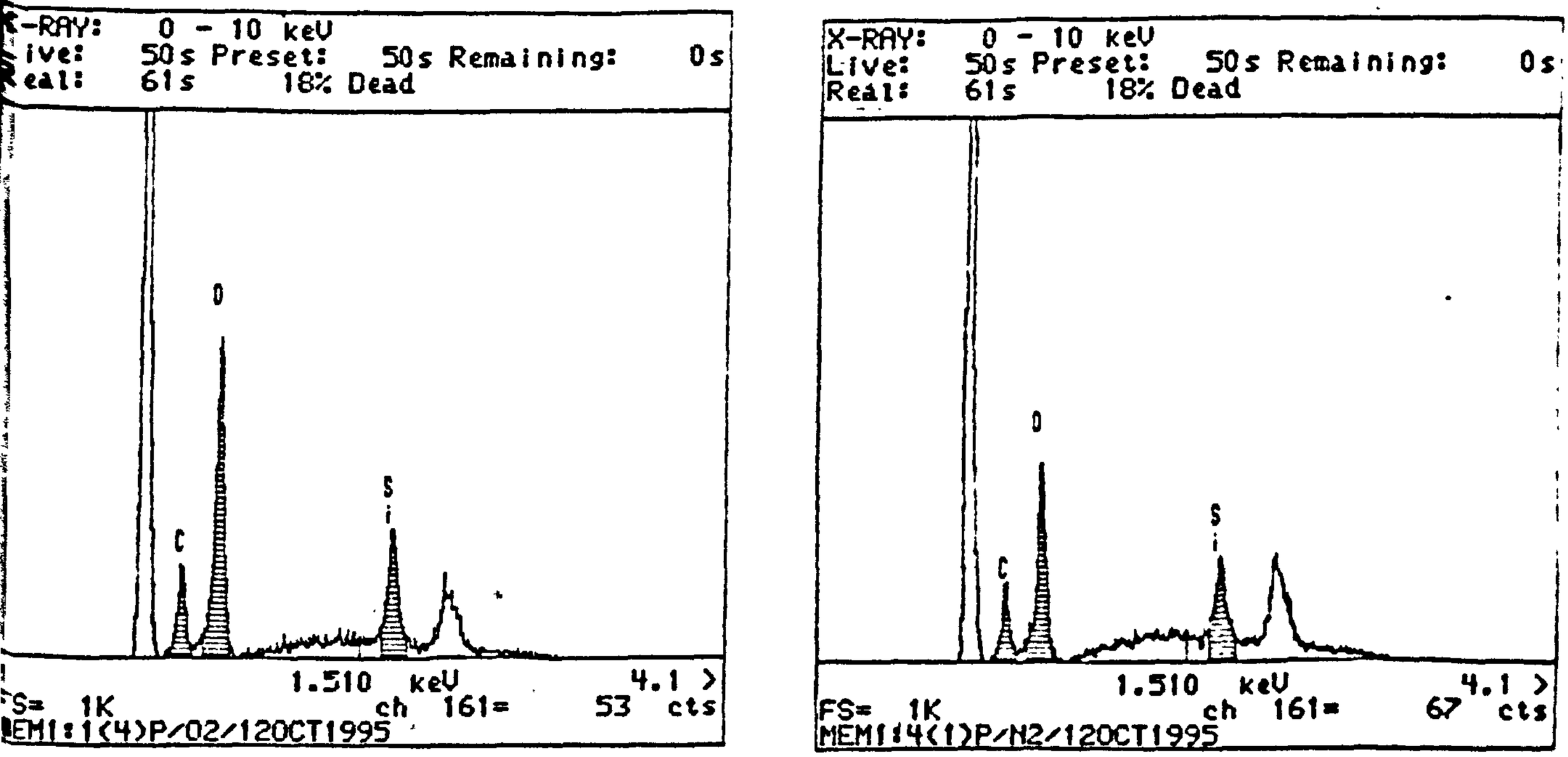


Figure 4.4 ATR - FTIR spectra of the PDMS treated with the CO_2-pulsed laser at nitrogen atmosphere at the wavelength of 9.58 μm (1043 cm^{-1}): sample (a) 5 pulse treated; (b) 10 pulses treated; (c) 15 pulses treated; (d) untreated PDMS.

of these groups are higher than under nitrogen, and the intense peak (1746 cm^{-1}) was not been observed under the nitrogen atmosphere. Therefore oxidation of groups on the surface of laser treated PDMS under a nitrogen atmosphere is very slight. We did not observe any nitrogen groups on these surfaces.

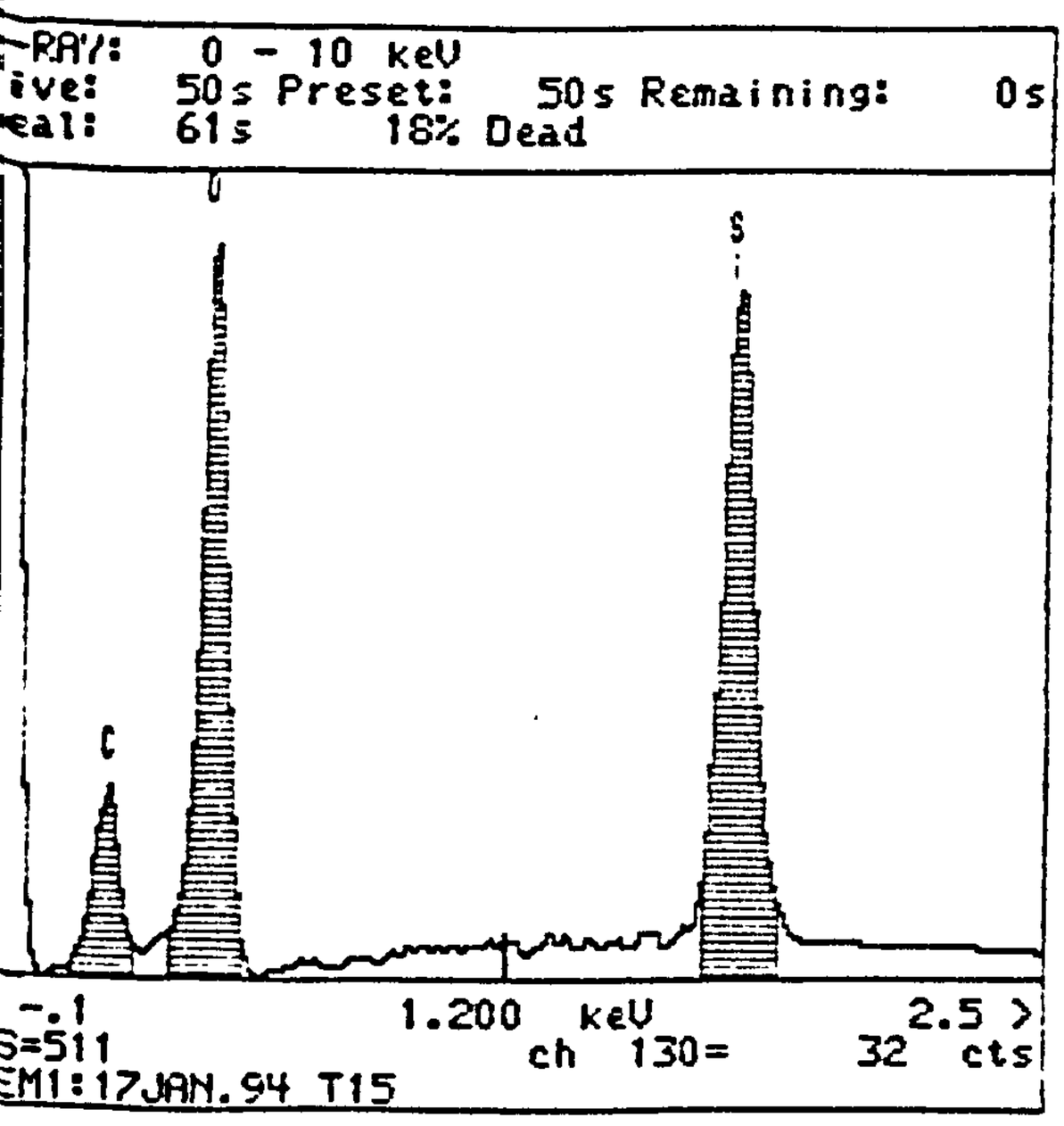
4.1.2 EDXA study

Energy dispersive X-ray analysis was used to measure the depth and homogeneity and O/Si ratio of the modified samples. Figure 4.5(a-d) shows the energy dispersive x-ray analysis of a PDMS surface which was treated by CO_2-pulsed laser at the wavelength of 9.58 μm (1043 cm^{-1}) at oxygen (32% O, 24% C, 43% Si) (a), nitrogen (26% O, 31% C, 41% Si) (b) and air (29% O, 29% C, 41% Si) (c) atmosphere and compared with untreated PDMS (d). EDXA analysis showed that all of the treated PDMS surfaces contain a higher ratio of O/Si than the base PDMS. The percentages of O, Si, C on the surface of the untreated PDMS were found to be 23%, 42%, 34% and under oxygen atmosphere, after 5 pulses were found to be 32%, 43%, 24% , respectively, which shows a higher percentage of oxygen on the surface of the treated PDMS compared to the untreated sample, which are consistent with the FTIR results. As can be seen the O/Si ratio in an oxygen atmosphere is higher than in air and in air is higher than nitrogen. This indicates that the oxidation reaction is the basic reactions for laser irradiation on the surface of PDMS.

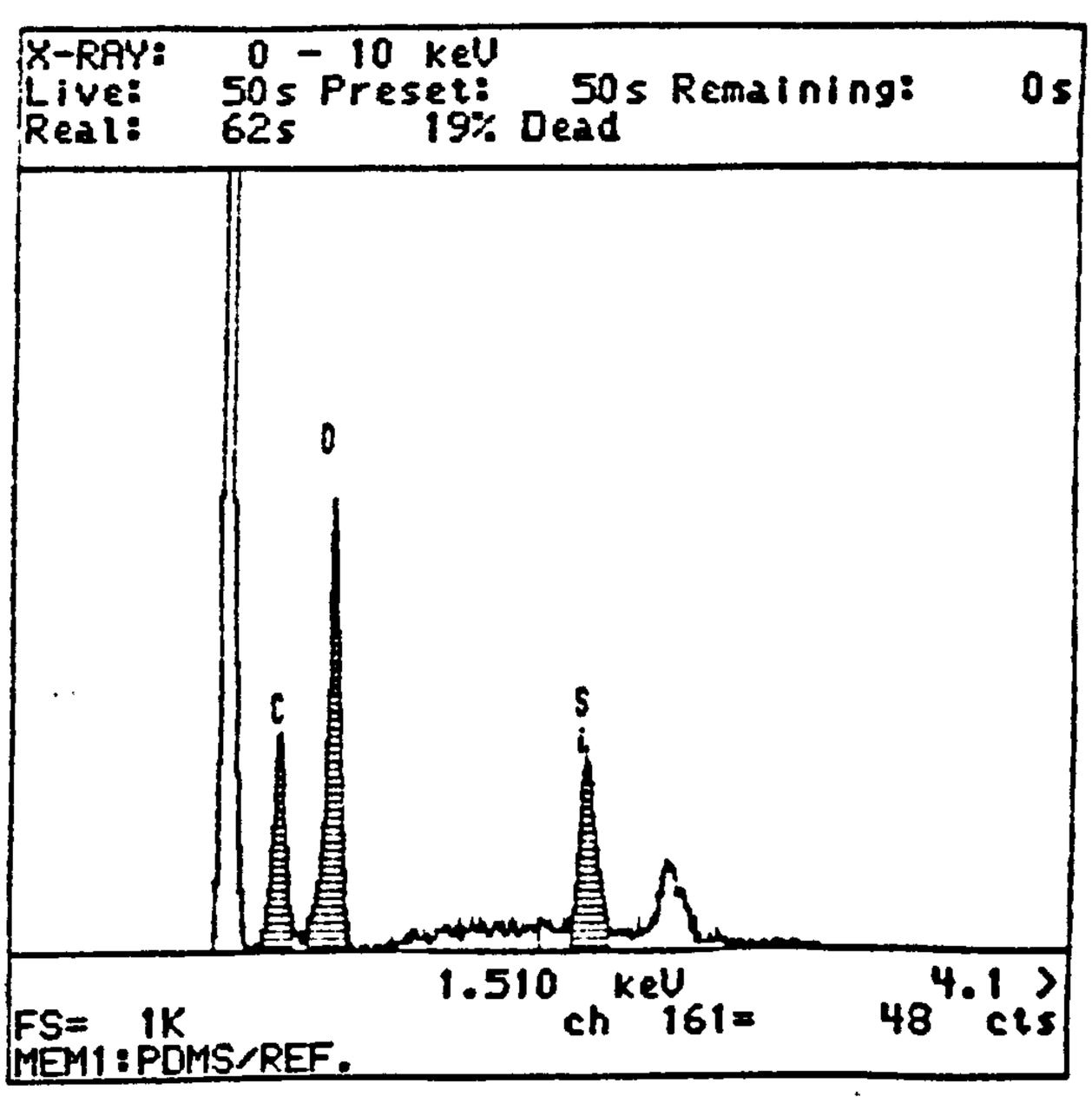


(a) (b)

Figure 4.5 EDXA analysis of PDMS treated with the CO_2-pulsed laser by 5 pulses at the wavelength of 9.58 μm (1043 cm^{-1}): (a) at oxygen atmosphere; (b) at nitrogen atmosphere.



(c)



(d)

Figure 4.5 EDXA analysis of PDMS treated with the CO_2-pulsed laser by 5 pulses at the wavelength of 9.58 μm (1043 cm^{-1}): (c) at air atmosphere; (d) untreated PDMS.

It was observed that the induced changes were reproducible. The EDXA analysis and ATR-FTIR spectra show that the CO_2-pulsed laser induced reactions and fragmentations on the surface of PDMS when the wavelength of the laser beam corresponds to the strong infrared absorption of PDMS. We believe that infrared laser induced surface modification of PDMS by vibrational excitation of -Si-O- bonds occurs through the infrared multiphoton dissociation (IRMPD) mechanism.

4.1.3 Infrared multiphoton dissociation mechanism (IRMPD)

Infrared radiation can be used efficiently when the wavelength of the beam corresponds with the vibrational energy of a specific bond of a molecule which participates in the polymerization or decomposition process and then a resonant situation is achieved in the reactions which leads to the cleavage of selected bonds. The most promising selective type of chemical reactions is the resonant interaction between infrared laser radiation and the vibrational mode of the structural groups, the dissociation of which results in photopolymerization or oxidation. In other words, the deposition of intense infrared radiation of the correct frequency can selectively and efficiently deposit tens of photons in isolated molecules and cause dissociation. This is an exciting breakthrough[81]. The process of IRMPD can be roughly divided into three regions. In the lowest region, the molecules are excited through discrete rotation-vibrational levels by intensity-dependent resonant absorption until the vibrational density of states becomes large enough for energy randomization to compete with absorption. In this" quasi-continuum", the molecules are pumped to higher and higher

levels by stepwise incoherent excitation. Once the molecules are excited over the dissociation barrier, decomposition competes with continued "up-pumping"[201]. We believe that infrared laser induced surface modification of PDMS occurs by vibrational excitation of -Si-O- bond through an infrared multiphoton dissociation (IRMPD) mechanism. According to this mechanism the radicals produced by laser irradiation may be transferred to the $-CH_3$ groups on the skeletal chain of the PDMS and these groups are then converted to the carbonate or other oxidized groups such as carbonyl and hydroxyl groups in the presence of oxygen. The schematic reactions of the oxidation pathway of the PDMS surface modification are presented in Scheme 1. Among many possible oxidation schemes tested to explain our experimental observation, that of reactions (I)-(III) give the most probable mechanism of the CO_2-pulsed laser-induced oxidation onto the PDMS. This mechanism is the same as the surface oxidation reactions of the polymer irradiation in the presence of oxygen.

Scheme 1. The schematic reactions of the laser oxidation pathway of the PDMS surface ; RR', RH and * and R°, R'°, H° denote PDMS (-O-Si-O-Si-), excited state and radicals respectively.

(I) Initiation:

$$R'R \longrightarrow (R'R)^*$$

$$(R'R)^* \longrightarrow (R')^\circ + R^\circ \quad \text{(energy transfer)}$$

(II) Propagation:

$$R^\circ + O_2 \longrightarrow ROO^\circ$$

$$R'^\circ + O_2 \longrightarrow R'OO^\circ$$

$$ROO^\circ + RH \longrightarrow ROOH + R^\circ \quad \text{(radical transfer)}$$

$$R'OO^\circ + RH \longrightarrow R'OOH + R^\bullet$$

$$ROOH \longrightarrow ROO^\circ + H^\circ$$

$$R'OOH \longrightarrow R'OO^\circ + H^\circ$$

(III) Termination:

$$ROOH \longrightarrow RC{=}O + H_2O \quad \text{(decomposition)}$$

$$R'OOH \longrightarrow R'C{=}O + H_2O$$

$$ROOH + O_2 \longrightarrow R\text{-}O\text{-}COO\text{-} + H_2O$$

$$R'OOH + O_2 \longrightarrow R'\text{-}O\text{-}COO\text{-} + H_2O$$

However, we cannot unambiguously conclude that these are the only reactions involved for the oxidation processes, because not all reactions have been identified.

4.1.4 Morphology of Laser Treated PDMS under Different Atmospheres

In Figure 4.6(a-c), examples of the SEM micrographs of CO_2-pulsed laser treated PDMS in the oxygen, air and nitrogen atmosphere are given and compared with that of an untreated one (Figure 4.6d). These Figures show the continuous and homogeneous porosity of the surface of treated samples [Figure 4.6(a-c)] in comparison with the unmodified sample (Figure 4.6d). Untreated PDMS has a smooth surface, which, after treating by laser, changes to a rough surface with micropores. The width of pores onto the surface of laser treated PDMS in an oxygen atmosphere at 10 pulses are about 1 - 10 μm, and the depth is about 1-5 μm [Figure 4.7(a,b)]. In other words, the laser treated PDMS structures appear to contain porosity in a similar size range and are uniform over all of the exposed, treated surface. As shown in Figure 4.6c the width of porosity at the surface of laser treated PDMS in nitrogen atmosphere is higher than the oxygen atmosphere, but the depth is lower than it. The morphological differences between oxygen and nitrogen atmosphere depends on the propagation reaction of the oxidation reaction mechanisms, as illustrated in Scheme 1. It seems that the interaction between the PDMS macromolecular chains and the photons in an oxygen atmosphere is higher than in a nitrogen atmosphere and this factor causes the different surface morphologies which we have obtained. It has been reported that the pore size and porosity and super-hydrophobicity have major effects in the design and production of synthetic vascular grafts[2,202,203,204].

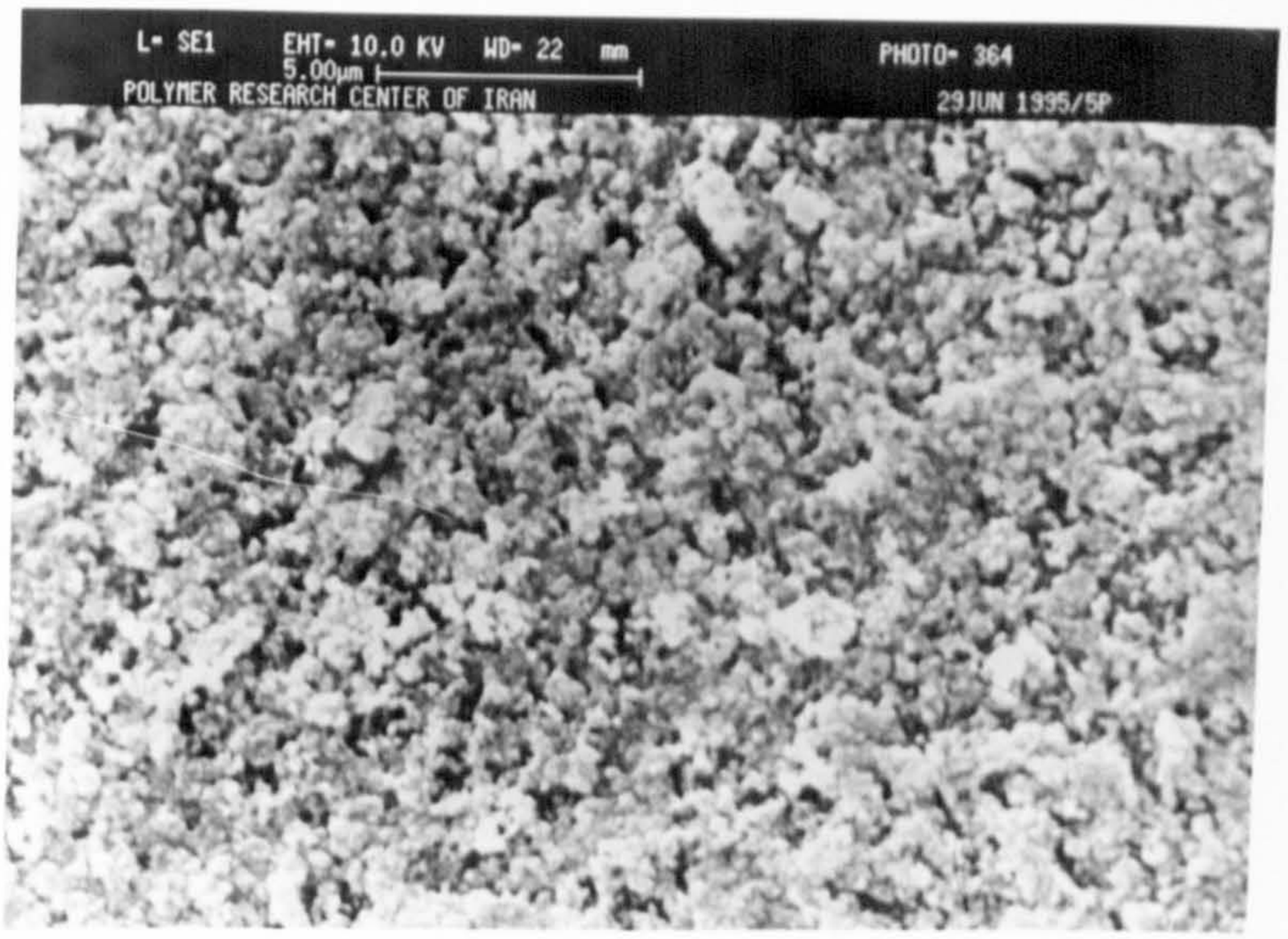


Figure 4.6 (a) SEM micrographs (magnification is 5000 x) of treated samples with the CO_2-pulsed laser by 5 pulses at wavelength of 9.58 µm (1043 cm^{-1}) at oxygen atmosphere.

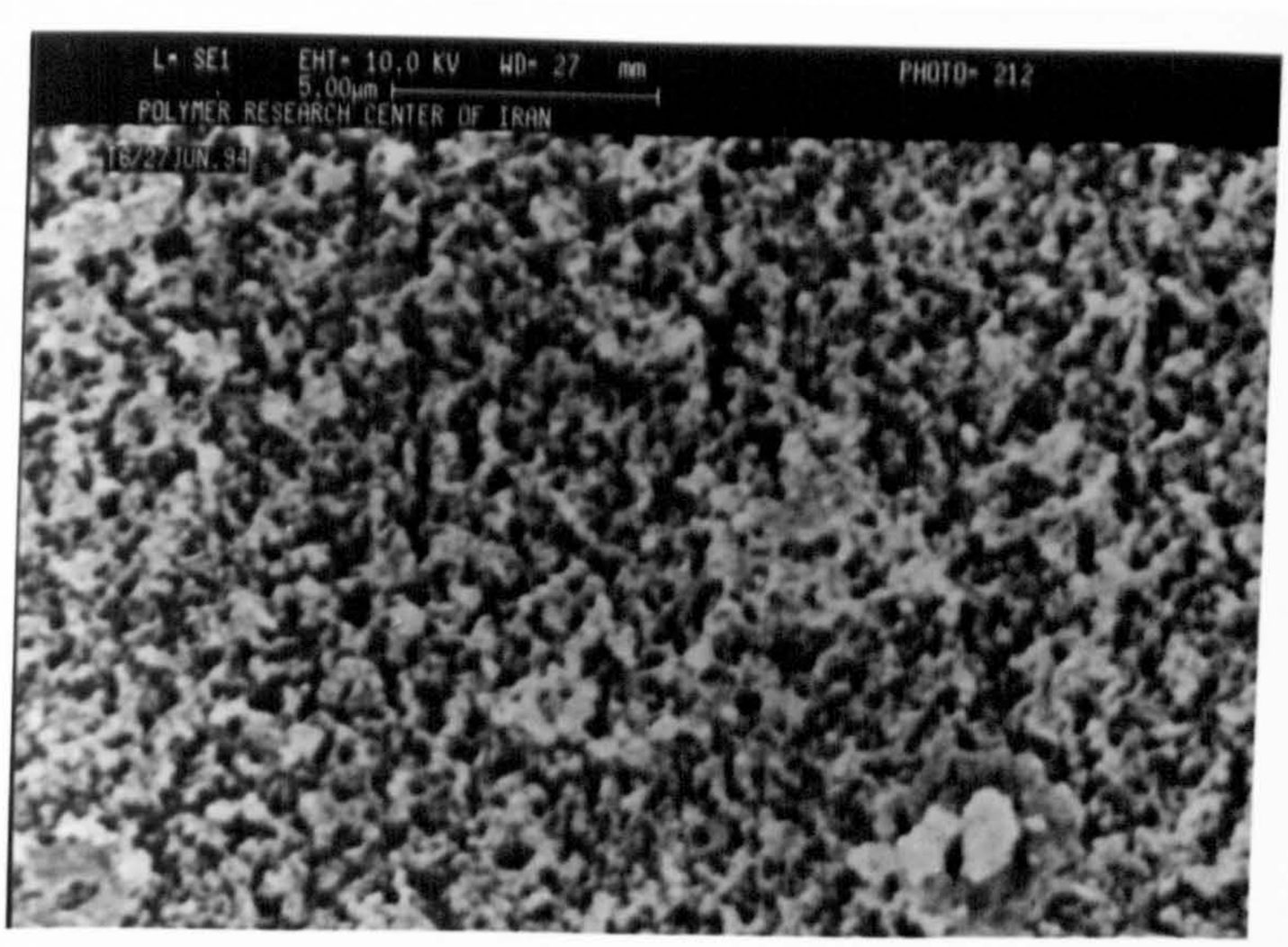


Figure 4.6 (b) SEM micrographs (magnification is 5000 x) of treated samples with the CO_2-pulsed laser by 5 pulses at wavelength of 9.58 μm (1043 cm^{-1}) at air atmosphere.

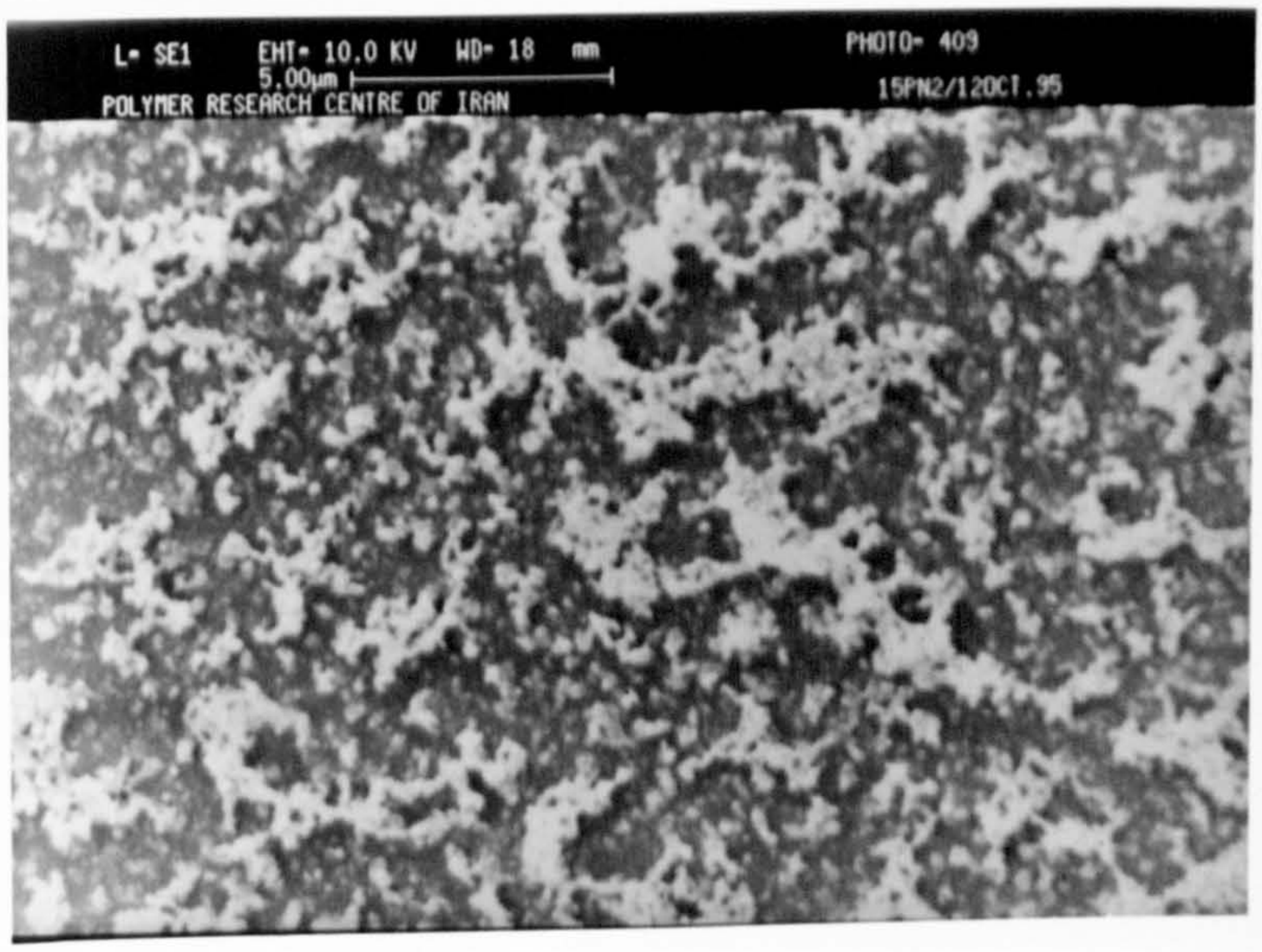


Figure 4.6 (c) SEM micrographs (magnification is 5000 x) of treated samples with the CO_2-pulsed laser by 5 pulses at wavelength of 9.58 μm (1043 cm^{-1}) at nitrogen atmosphere.

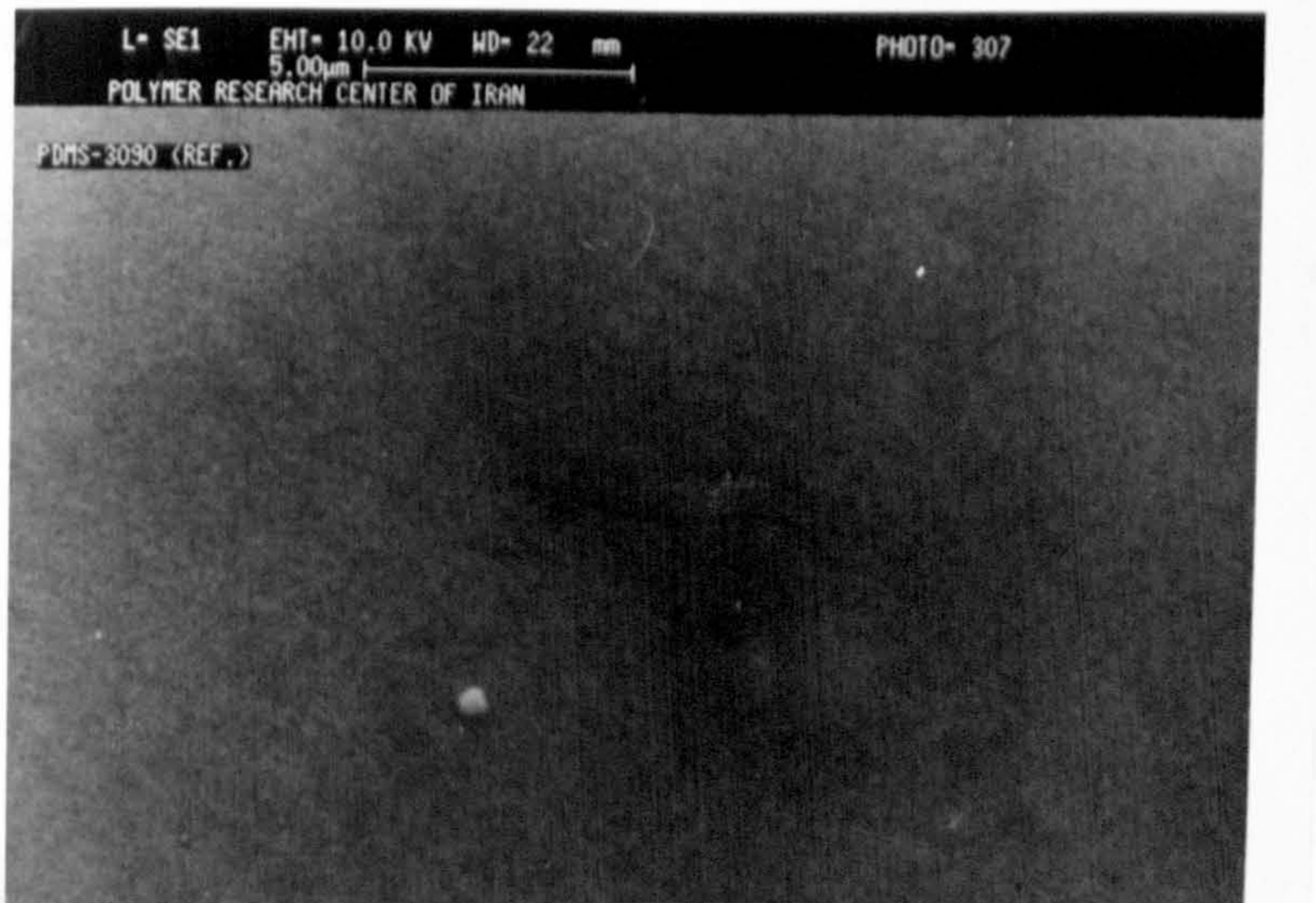
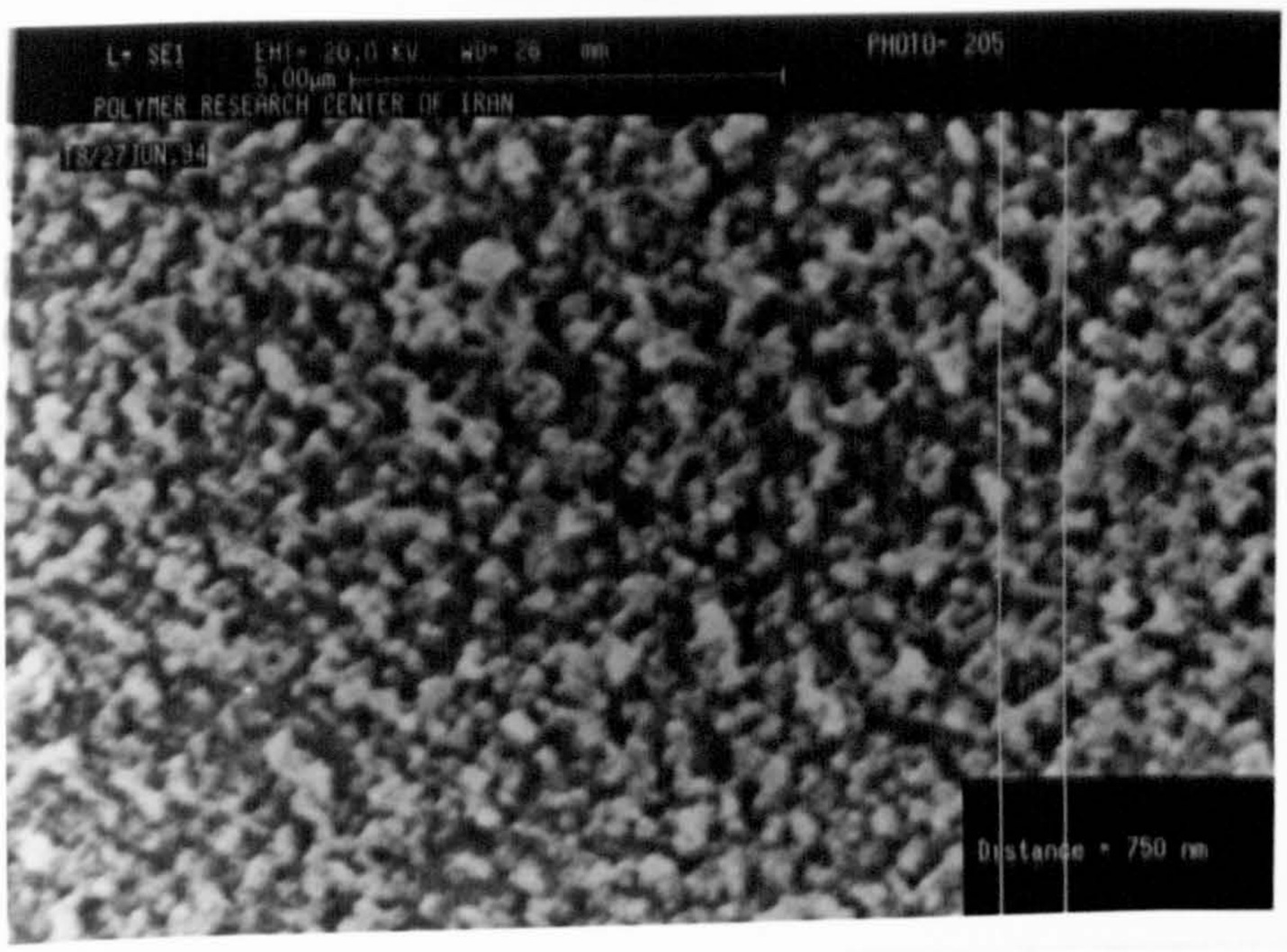
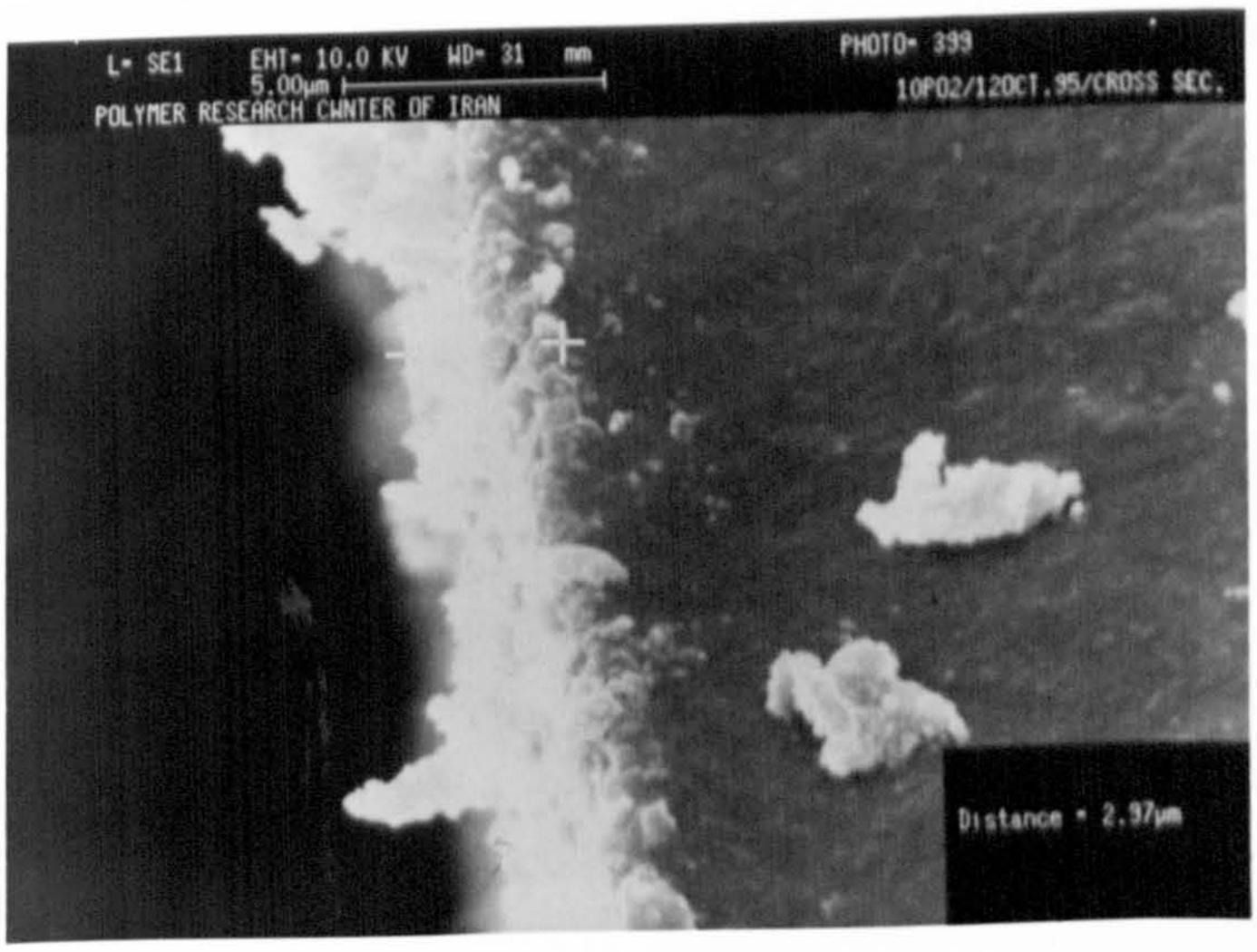


Figure 4.6 (d) Untreated PDMS (magnification is 5000 x).



(a)



(b)

Figure 4.7 SEM micrographs of the surfaces of: (a) treated PDMS at 9.58 µm (1043 cm^{-1}) wavelength with 10 laser pulses; (b) cross section of the treated PDMS.

4.1.5 Effect of pulse number

The variation of the O/Si intensity ratios determined from EDXA analysis is given in Figure 4.8 as a function of the pulse number. As can be seen in this figure, by increasing the pulse number, the O/Si ratio on the treated surface is increased up to 5 pulses after which this ratio tends to a plateau state. The increased O/Si intensity ratio is evidence for oxidized groups formed by laser treatment. We have at present no clear explanation for the appearance of the plateau in Figure 4.8 but it seems likely that the oxidized groups formed upon laser treatment could be ablated by further CO_2-pulse laser treatment.

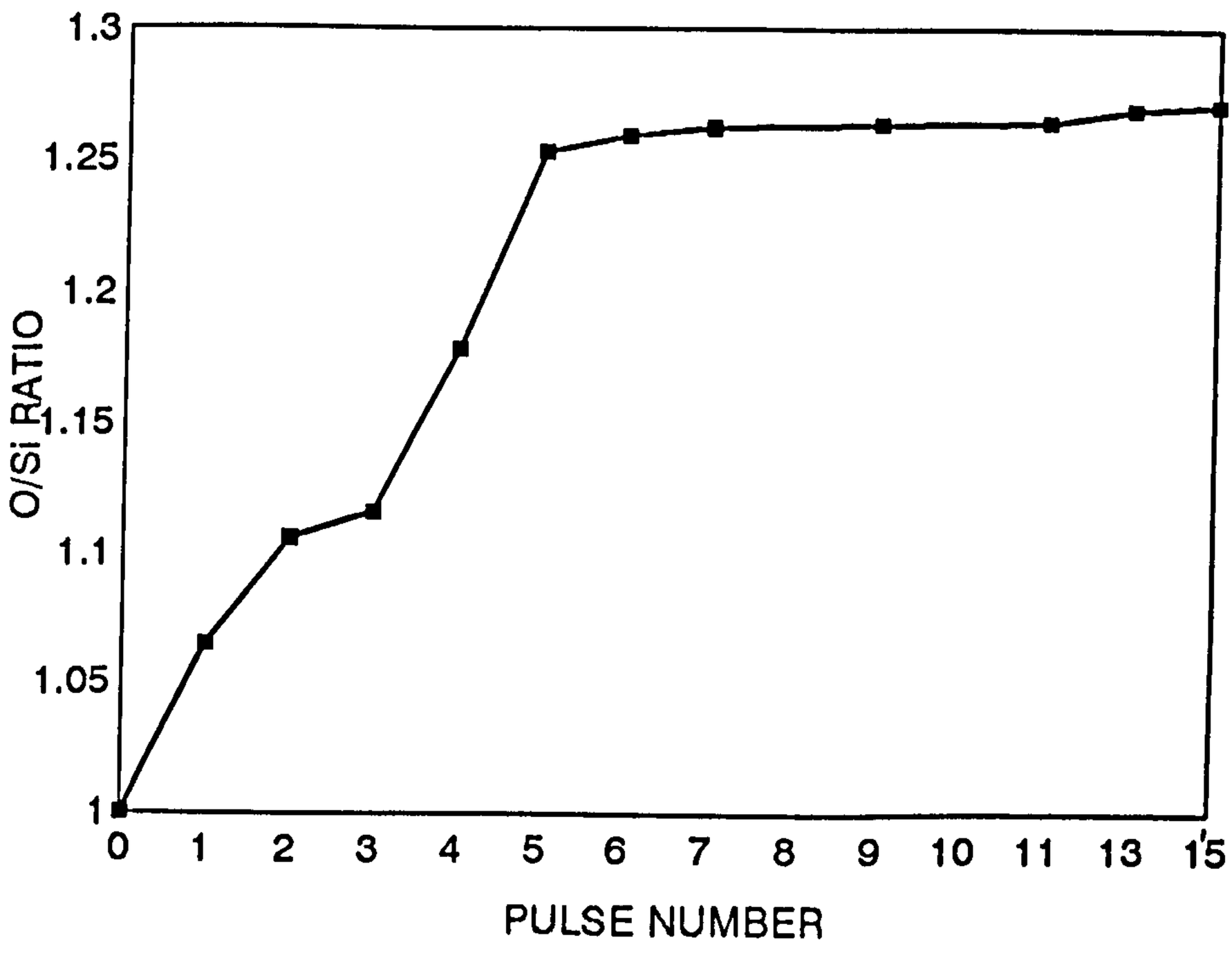


Figure 4.8 Dependence of O/Si ratio of treated samples on pulse number.

Figure 4.9 shows the increasing of pulse number on the surface of PDMS. When the surface of PDMS is exposed to only a single pulse of the laser beam, the surface becomes rough and covered with micropores; by increasing the pulse number to between 5-10 pulses, this porosity becomes periodic and homogeneous (Figure 4.6a) and, as will be discussed in section (4.3.1), this morphology has very good effect on blood compatibility. When the pulse number is increased, to 15 pulses, the homogeneity of porosity drops and, at 15 to 20 pulses, the surface changes to a new morphology which has very little porosity as shown in Figure 4.9.

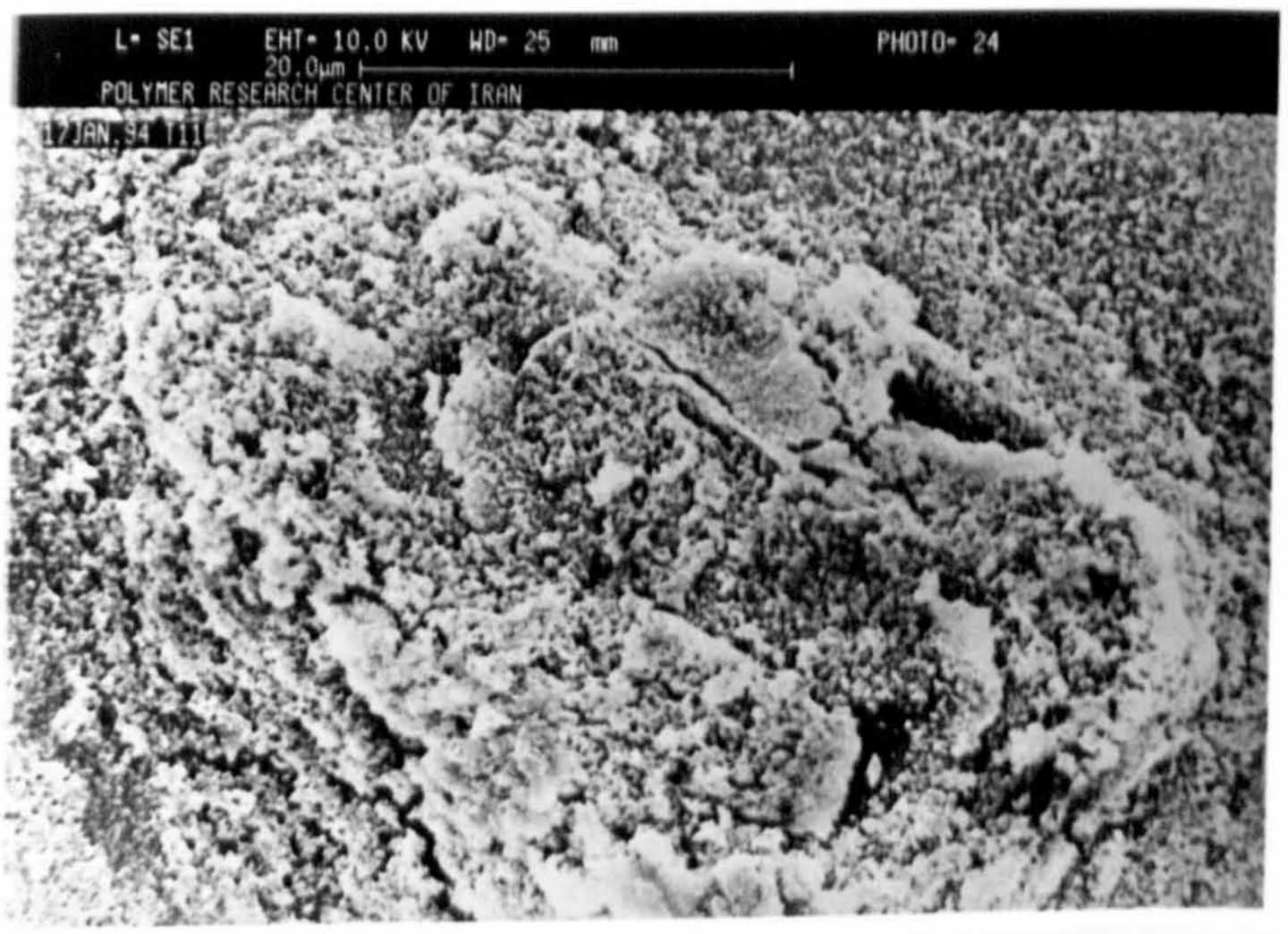


Figure 4.9 SEM micrographs (magnification is 1000 x) of treated samples with the CO_2 - pulsed laser by (a) 15 pulses at wavelength of 9.58 μm (1043 cm^{-1}) at oxygen atmosphere.

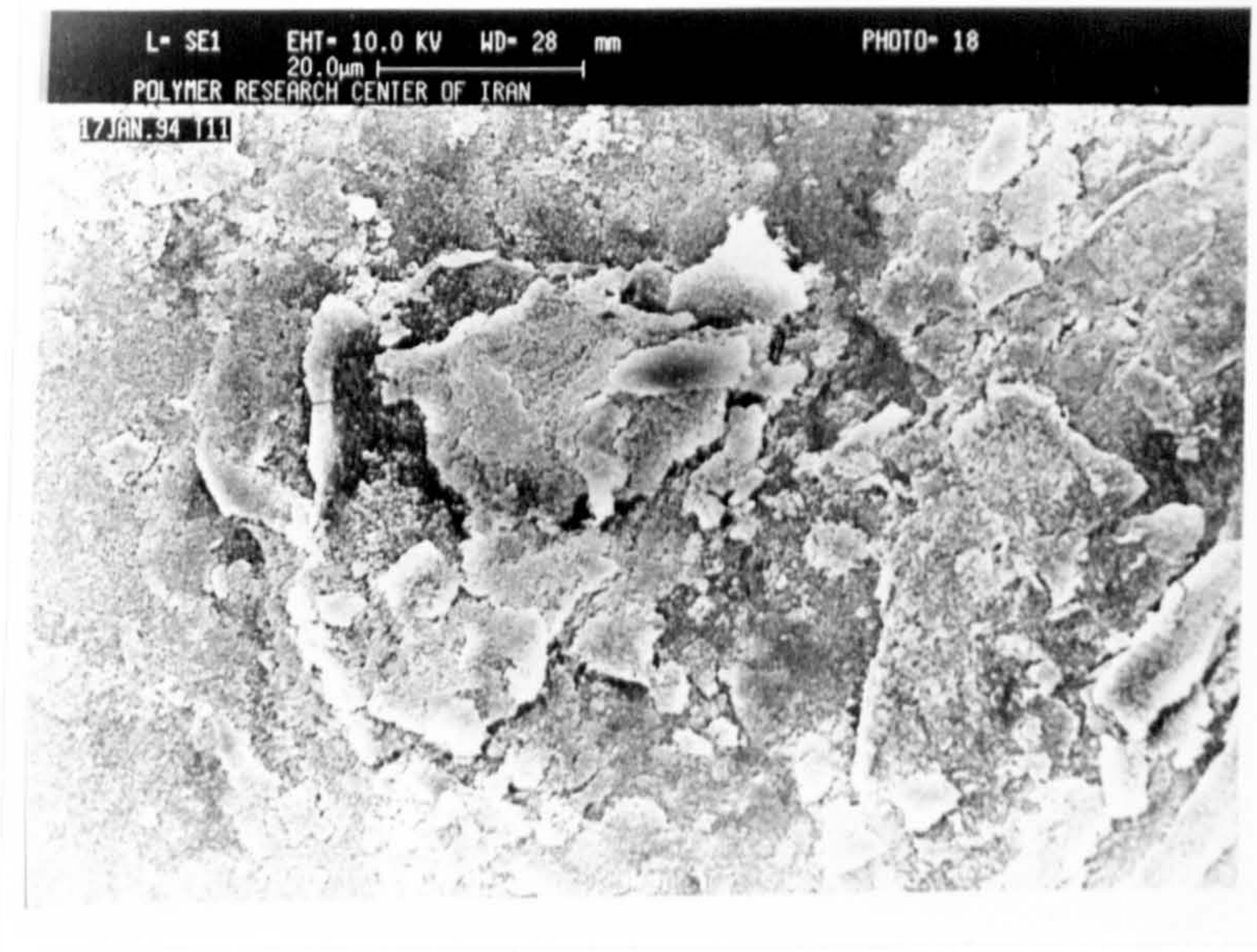
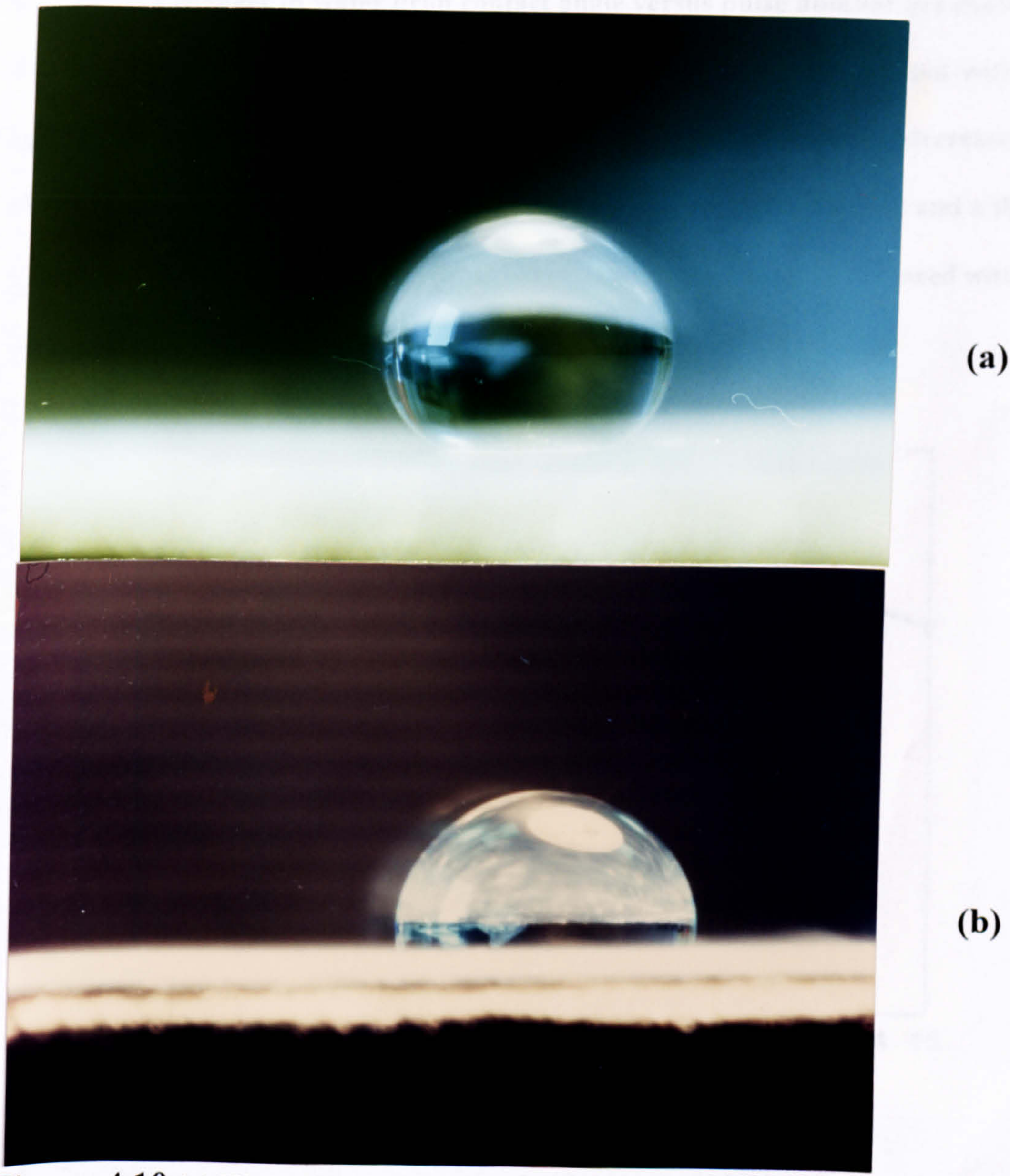


Figure 4.9 **SEM micrographs (magnification is 500 x) of treated samples with the CO_2 - pulsed laser by: (b) 20 pulses, at wavelength of 9.58 μm (1043 cm^{-1}) at oxygen atmosphere.**

This phenomenon is due to further ablation of the surface as the pulse number is increased. After such drastic treatment, surface ablation of the PDMS occurs to the extent of breaking many of the polymer chains. This result demonstrates the advantage of being able to control the laser beam in a pulsed method. Only between 5-10 pulses are required for the optimal modification of the surface of the PDMS.

4.1.6 Wettability

The variation in hydrophobicity of the treated sample is shown in Figure 4.10a and is compared with the untreated sample in Figure 4.10b.



(a)

(b)

Figure 4.10 (a) Water drop contact angle of the treated PDMS by 5 pulses at the wavelength of 9.58 μm (1043 cm^{-1}); (b) untreated PDMS.

PDMS exhibits hydrophobic behaviour and poor wettability. The virgin PDMS surface film has a water drop contact angle of 105°. After treatment of PDMS by the CO_2-pulsed laser, by only one pulse, the water drop contact angle changed to give a value of 170°. The changes in water drop contact angle versus pulse number are shown in Figure 4.11. As can be seen in this Figure, the contact angle is increased with the increasing pulse number up to 5 pulses above which the contact angle decreased. It means that the surface property of the treated samples has been changed and a that a surface was obtained which behaves as a highly hydrophobic one as compared with the unmodified PDMS.

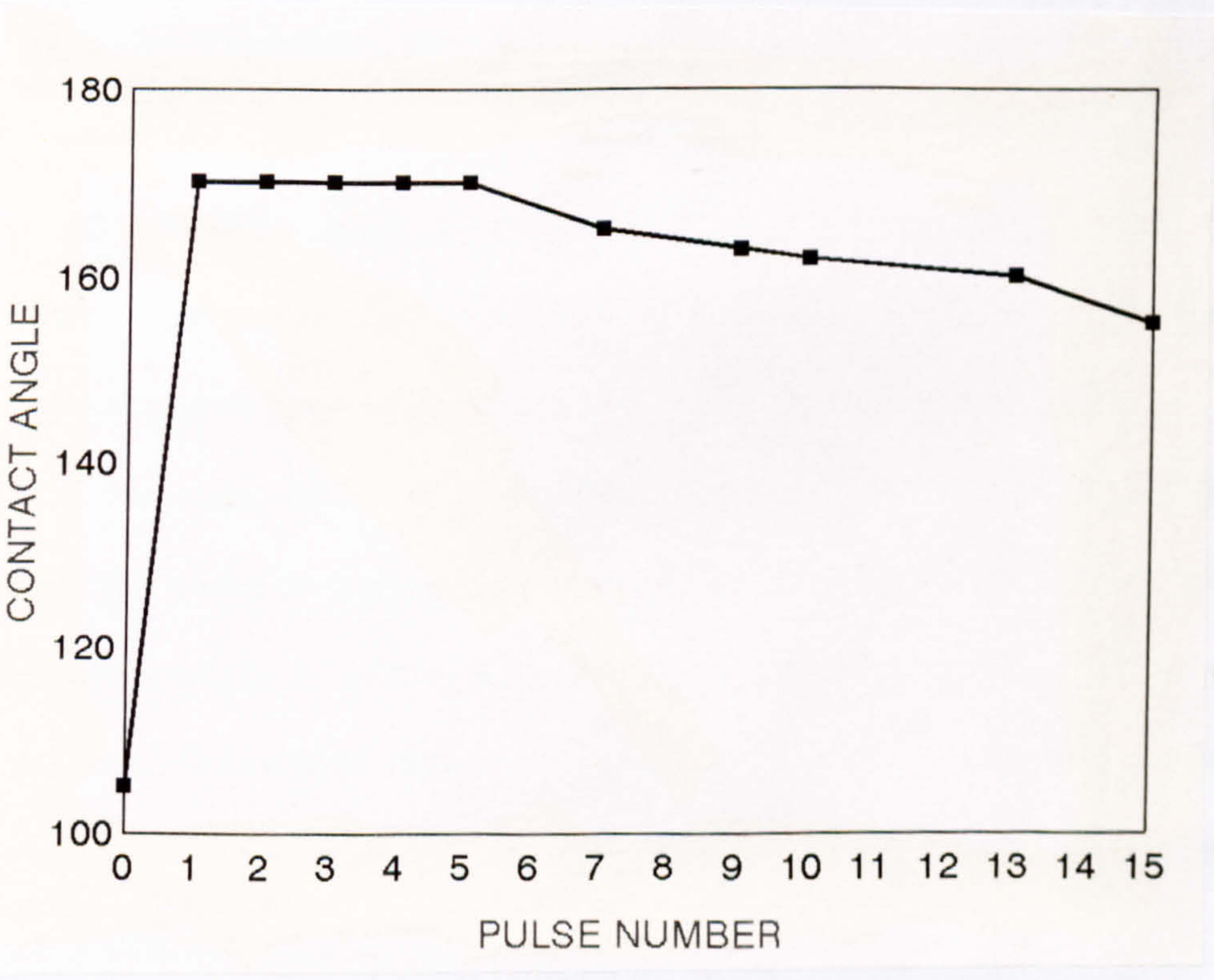


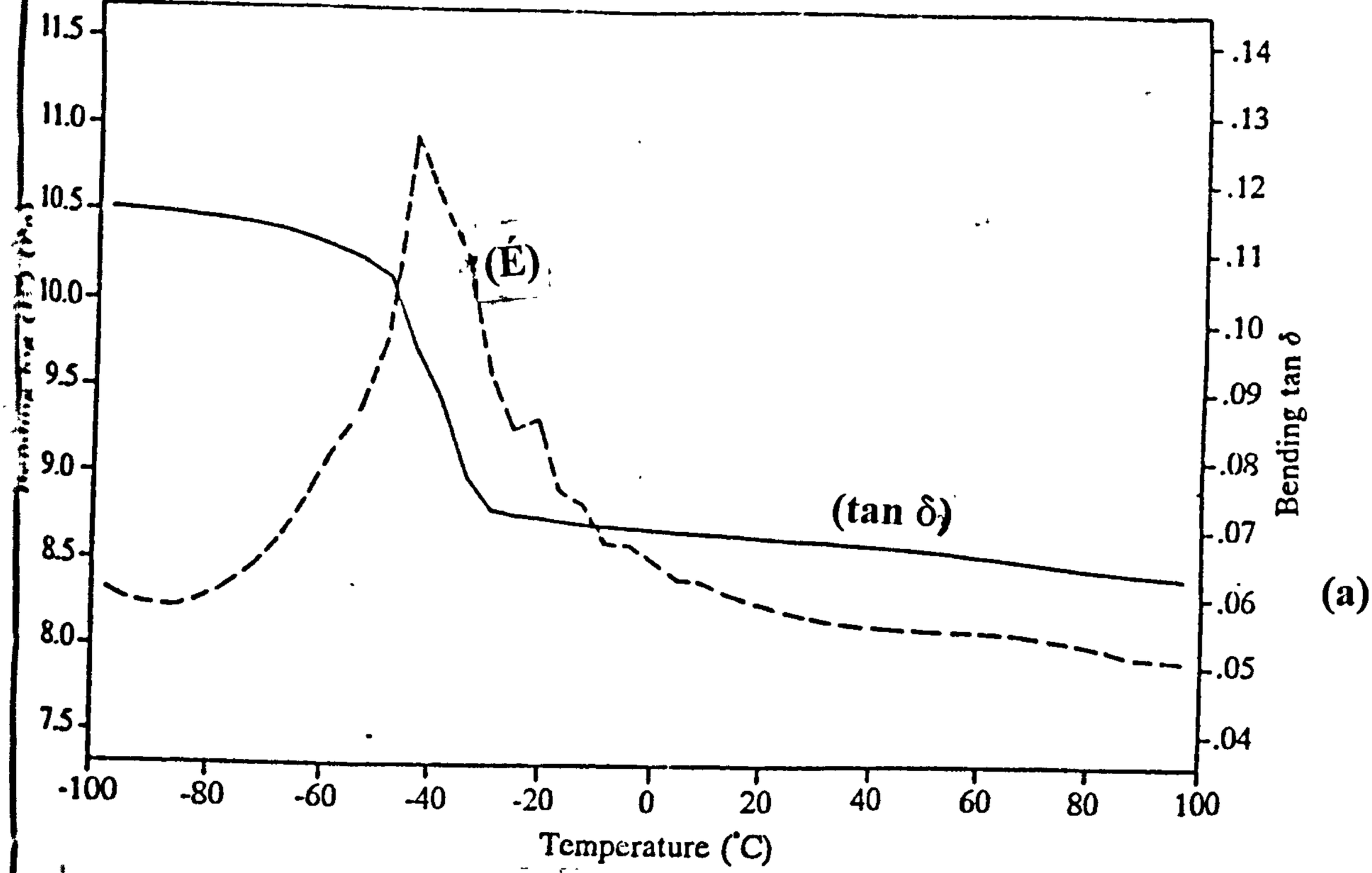
Figure 4.11 Changes of the water drop contact angles of the treated PDMS samples with the pulse number.

As reported in other studies,[157] it is believed that this unexpectedly high contact angle phenomenon in surface treated polymers are controlled by a large number of different interactions, both chemical and physical, involving modified chains and the environment, surface and subsurface molecules and interactions among the treatment-introduced functional groups and the morphology of the polymer. We assume that the surface morphology and surface functionalities of PDMS play a significant part in the formation of the highly hydrophobic surface. Thus, by increasing the pulse number from 10 to 20, known to affect the morphology (Figure 4.9), the wettability increases. As discussed in section (4.1.4), use of 5 to 10 pulses, produces a PDMS surface with a homogenous microporosity. This phenomena has a major effect on wettability and, as a consequence, on blood compatibility. In the literature it has been reported[2] that the vascugraft prothesis, using an expanded PTFE material (Gore Tex) is also hydrophobic and gives a high contact angle measurement; GoreTex, with its inherently low surface tension, gives advancing water contact angle values of around 116° [205,206]. However, some work on the nature of the surface groups on performance of materials has also been reported,[2] for example that the presence of surface carbonate groups, as produced by PDMS laser treatment, which enriches the surface oxygen content, causes the polymer surface to become relatively *more* hydrophobic. It can only be assumed that the morphology (microporosity) of the surface is more important than the nature of the chemical functional groups, although the latter must also play a part in the overall biocompatibility (or otherwise) of a surface.

4.1.7 Dynamic mechanical thermal analysis

The weak point in using high energy radiation, such as gamma or electron beams as the excitation source for surface modification, is that the bulk mechanical properties of the substrate may also change[98,207]. However, this potential shortcoming can be avoided by using the CO_2- pulsed laser source, providing the beam is tuned to the absorption maxima of substrate at the surface[6,7]. To verify this the laser treated PDMS samples were examined by dynamic mechanical thermal analysis (DMTA). The storage modulus (É) and the loss tangent (tan δ) and their trend of variation with temperature are shown and compared for laser treated PDMS and unmodified PDMS respectively in Figure 4.12(a-b).

It can be seen that the storage modulus for the treated PDMS is comparable with that of the control. Also tan δ_{max} has appeared within the same temperature region for both modified and unmodified samples. As (É) and the loss tangent (tan δ) are related to the bulk structure of the rubber, these results show an insignificant change in the bulk mechanical properties of the laser treated PDMS. It can be concluded that the bulk structure and, therefore, the mechanical properties of the laser-treated samples have remained intact. Irradiating a polymeric material with a continuous laser beam heats the surface and may damage both the surface and the bulk material. In contrast, pulsed lasers allow short exposure times and less thermal damage by optimizing the time intervals between pulses (repetition rate), laser fluency and pulse number[9,92,107].



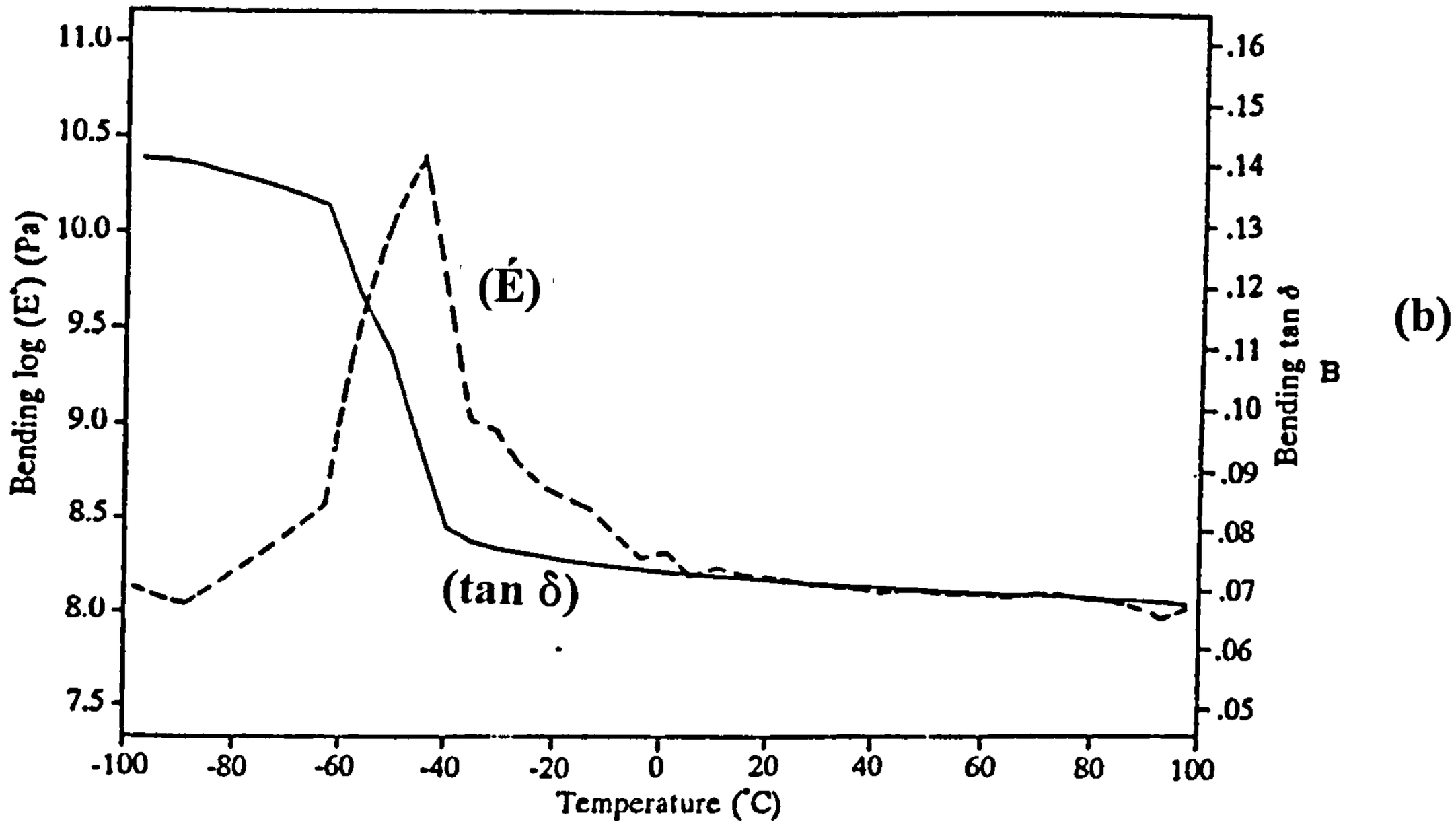


Figure 4.12 Variation of storage modulus (É) and the loss tangent (tan δ) with the temperature for: (a) PDMS treated with the CO_2-pulsed laser by 5 pulses at 9.58 μm (1043 cm^{-1}) wavelength; (b) untreated PDMS.

4.1.8 Friction coefficient

If there is a significant frictional resistance between the device surface and the mucous membrane of the body during the insertion or removal of the device, mechanical damage will occur on the mucous membrane[1].

After the surface of the silicone was laser-treated, the hydrophobic surface became very slippery in a dry state. To quantitatively characterize the surface slipperiness, the friction coefficient of the surface of PDMS, treated in oxygen, nitrogen and air atmospheres, were determined. The observed results are shown in Figure 4.13.

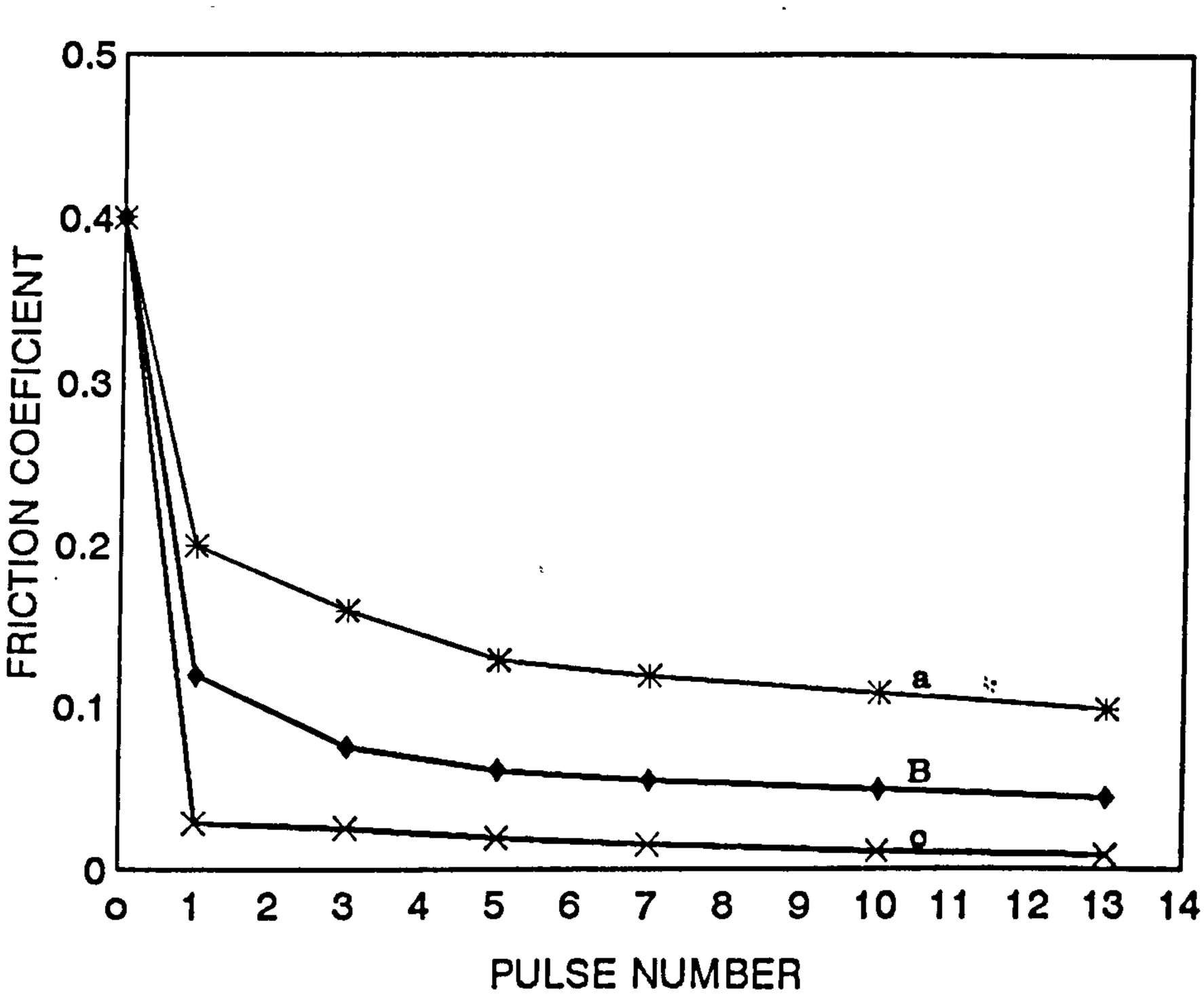


Figure 4.13 Changes of the friction coefficients of the treated PDMS samples with the pulse number at different atmosphers: (a) nitrogen; (b) air; (c) oxygen.

As is apparent, the friction coefficient of the untreated silicon is 0.4 but drastically decreases when the surface is laser irradiated, even if only a single pulse was delivered to the silicone surface. The friction coefficient (starting force $a\mu_c$) is 0.011 when the silicone surface was treated by 10 pulses in an oxygen atmosphere. The trend of decrease of friction coefficient of the laser treated PDMS in air and nitrogen atmosphere versus pulse number is the same as in oxygen, but in air is lower than oxygen and in nitrogen atmosphere is lower than air. These differences depends both on the morphology which appear on the surface of treated PDMS under different atmospheres and the nature of the chemical groups on the surface of PDMS ; of these the morphology changes have the most significant effect on decreasing of the friction coefficient.

4.1.9 Conclusion

Our results from CO_2- pulsed laser treated onto the surface of PDMS indicate the potential of using CO_2- pulsed laser to induce oxidation and also structuring onto the surface of PDMS, without the need for a photosensitizer, that changes the property of the PDMS surface's chemical and physical properties. The modified surfaces have uniform porosity. PDMS is surface modified by CO_2- pulsed laser providing it is irradiated by pulses having a wavelength where the PDMS has a strong IR absorption. We believe that the CO_2- pulsed laser induced modification on the surface of PDMS at 9.58 μm (1043 cm^{-1}) occurs by the vibrational excitation of -Si-O- bond through infrared multiphoton dissociation mechanisms (IRMPD). The modified PDMS contains

carbonate and the other oxidized groups, such as hydroxyl and carboxyls, which enrich the oxygen content of the surface. Treated samples showed significant variation in hydrophobicity and the water drop contact angle increases up to 175° and is found to be dependent upon the number of irradiated laser pulses. The thickness of the modified surfaces of PDMS is about 1 μm, hence the bulk structure and therefore the mechanical properties of the laser treated samples have remained intact.

4.2 Grafting of Acrylate Monomers onto PDMS

Acrylamide (AAm), 2-hydroxyethylmethacrylate (HEMA) and hydroxyethylmethacrylate phosphatidyl choline (HEMAPC) (Table 1) were grafted onto the polydimethylsiloxane surface. PAAm, PHEMA and PHEMAPC are known as hydrogels with high hydrophilicity and good biomedical properties[208] . However, their main disadvantages as stand alone polymers are their weak mechanical properties when swollen in water[135]. Until recently, the major obstacle to the use of hydrogels has been this general lack of strength. This problem may be circumvented by the grafting of thin layers of the hydrogel polymers to polymer substrates having mechanical and transport properties more consistent with the biomedical applications[209]. Modifying the polymer surface by graft copolymerization has also been made possible by free radicals or peroxides generated by laser treatment[30].

A silicone sheet was first treated by a CO_2- pulsed laser under oxygen, nitrogen and air atmospheres to introduce peroxides onto the surface. Such polymeric peroxides were capable of initiating graft polymerization onto the PDMS. The concentration of

peroxides introduced to the surface was determined using the iodide method. Graft

Table 1 Chemical formula of acrylate monomers which were used for grafting onto the PDMS surface.

Monomer	Formula
Acrylamide	$CH_2 = CH - \overset{\overset{O}{\|}}{C} - NH_2$
HEMA	$CH_2 = CH - \overset{\overset{O}{\|}}{C} - O - CH_2 - CH_2 - OH$
HEMAPC	$CH_2 = CH - \overset{\overset{O}{\|}}{C} - O - CH_2 - CH_2 - O - \overset{\overset{O}{\|}}{\underset{\underset{O^-}{\|}}{P}} - O - CH_2 - CH_2 - N^+Me_3$

polymerization was allowed to proceed by heating the aqueous solution of the monomer together with the immersed PDMS pre-irradiated with the CO_2- pulsed laser. The graft polymerization of acrylate monomer did not occur irrespective of heating unless carried out under deaeration. The surface of control, laser treated and polyacrylate

modified PDMS rubber were characterized by ATR-FTIR and scanning electron microscopy (SEM), and staining techniques and dispersive X-ray analysis (EDXA).

4.2.1 Peroxide Formation

As reported in the above section, the oxidation reactions result from the laser irradiation at 9.58 μm (1043 cm^{-1}) wavelength. The concentration of peroxides formed in the surface layer of PDMS by CO_2 laser treatment at 9.58 μm by various pulse number is shown in Table 2.

As can be seen, peroxides are formed to a maximum concentration of $7x10^{-7}$ mol cm^{-2} by only one pulse of the CO_2- laser, and then decreased by increasing the pulse number of the CO_2- laser. It is supposed that the number of reaction sites to form the peroxides onto the PDMS film are reduced as the laser irradiation proceeds. Accordingly, the decomposition of peroxides by further laser irradiation overcomes the formation of new peroxides, resulting in the maximum amount of peroxide being formed after a single pulse irradiation; other oxygen groups on the surface of PDMS such as carbonyl, hydroxyl, and carbonate were also formed. As mentioned above, we believe that the infrared laser induced surface treatment of PDMS by vibrational

Table 4.2 Concentration of peroxides formed onto the surface layer of PDMS by CO_2-pulsed laser at 9.58 μm (1043 cm^{-1}) versus pulse number.*

Pulse number	Peroxide (mol cm^{-2})
1	7×10^{-7}
3	6.23×10^{-7}
5	6.09×10^{-7}
7	5.78×10^{-7}
10	5.47×10^{-7}
15	5.11×10^{-7}
20	4.20×10^{-7}

* Peroxides determined iodometrically.

excitation of the - Si - O - bond is through an infrared multiphoton dissociation (IRMPD) mechanism.

The schematic reactions of the laser oxidized pathway of the PDMS surface are presented below. RR' , RH, and (*) and R° , R'° , H° denote PDMS (-O-Si-O-Si-), the excited state and radicals respectively.

According to this mechanism, the -Si-O- bondis transformed into a vibrational excitation state by equation (1).

$$(R'R) \text{ ----------> } (R'R)^{*} \quad (1)$$

Radicals produced by dissociation of the excited molecules as equation (2).

$$(R'R)^{*} \text{ ----------> } (R)^{\circ} + (R')^{\circ} \quad (2)$$

These radicals immediately react with oxygen and peroxide radicals were produced, equation (3), (4).

$$(R)^{\circ} + O_2 \text{ ----------> } ROO^{\circ} \quad (3)$$

$$(R')^{\circ} + O_2 \text{ ----------> } R'OO^{\circ} \quad (4)$$

Then hydrogen abstraction was carried out and hydroperoxide formed as equations (5) and (6).

$$ROO^{\circ} + RH \text{ ----------> } ROOH + R^{\circ} \quad (5)$$

$$R'OO^{\circ} + RH \text{ ----------> } R'OOH + R^{\circ} \quad (6)$$

4.2.2 Surface Graft Polymerization

It should be emphasized that it is critical to exclude oxygen from the silicone interior as much as possible *prior* to the graft polymerization process, when the acrylate monomer is to be graft-polymerized onto the surface of the silicone. If the degassing of

the silicone was incomplete, graft polymerization of acrylate monomer onto the silicone surface did not occur. This is in marked contrast with the graft polymerization of acrylonitrile and methacrylic acid on uv-treated polyethylene[17] and grafting of acrylic acid onto a corona-treated polyethylene surface[18], where such prior degassing was not essential.. This implies that, in contrast to the other materials, the silicone contains plenty of dissolved oxygen, which is well known to act as a powerful inhibitor of radical graft polymerizations. Moreover, no graft polymerization took place, if the CO_2 laser treated PDMS was not heated to higher than 50°C, indicating that the thermal decomposition of the surface peroxides is essential for the initiation of graft polymerization. No graft polymerization was observed with untreated silicone.

As illustrated above, the hydroperoxides were decomposed by heating, as shown in equations (7) and (8).

$$ROOH \text{ ----------> } RO^{\circ} + {}^{\circ}OH \qquad (7)$$

$$R'OOH \text{ ---------> } R'O^{\circ} + {}^{\circ}OH \qquad (8)$$

The produced radicals will participate in the initiation of the grafting reaction according to the equations (9) and (10). [M, denotes the AAm, HEMA and HEMAPC.]

$$RO^{\circ} + M\ (AAm) \text{ ----------> } ROOM^{\circ} \qquad (9)$$

$$R'O^{\circ} + M\ (AAm) \text{ ----------> } R'OOM^{\circ} \qquad (10)$$

The propagation of the graft polymerization onto the surface of PDMS was carried out by equations (11) and (12).

$$ROOM^{\circ} + nM \text{ ----------> } ROO(M)_{n+1} \text{ grafted polymer} \quad (11)$$

$$R'OOM^{\circ} + nM \text{ ---------> } R'OO(M)_{n+1} \quad (12)$$

This causes a promoted grafting reaction. On the other hand, hydroxyl radicals formed by the equations (7) and (8) contribute to the formation of peroxides in the water medium, according to equation (13), and to the formation of homopolymer by equation (14).

$$2^{\circ}OH \text{ ----------> } H_2O_2 \quad (13)$$

$$^{\circ}OH + M \text{ (AAm) ----------> homopolymer} \quad (14)$$

The formation of homopolymer is detrimental since, in experimental work some homopolymer formed in the solution was then deposited onto the surface of PDMS. Therefore it is necessary to remove this homopolymer from the surface and this done by achieved by Soxhlet extraction of the grafted PDMS, using distilled water.

4.2.3 ATR-FTIR study of the AAm grafted onto PDMS

After Soxhlet extraction of the grafted PDMS in distilled water for 72 h to remove the homopolymer from the PDMS surface, the samples were dried and the

ATR-FTIR spectra was recorded. An example of the attenuated total reflectance fourier transform infrared (ATR-FTIR) spectra of the PDMS surfaces grafted with AAm are presented in Figure 4.14.

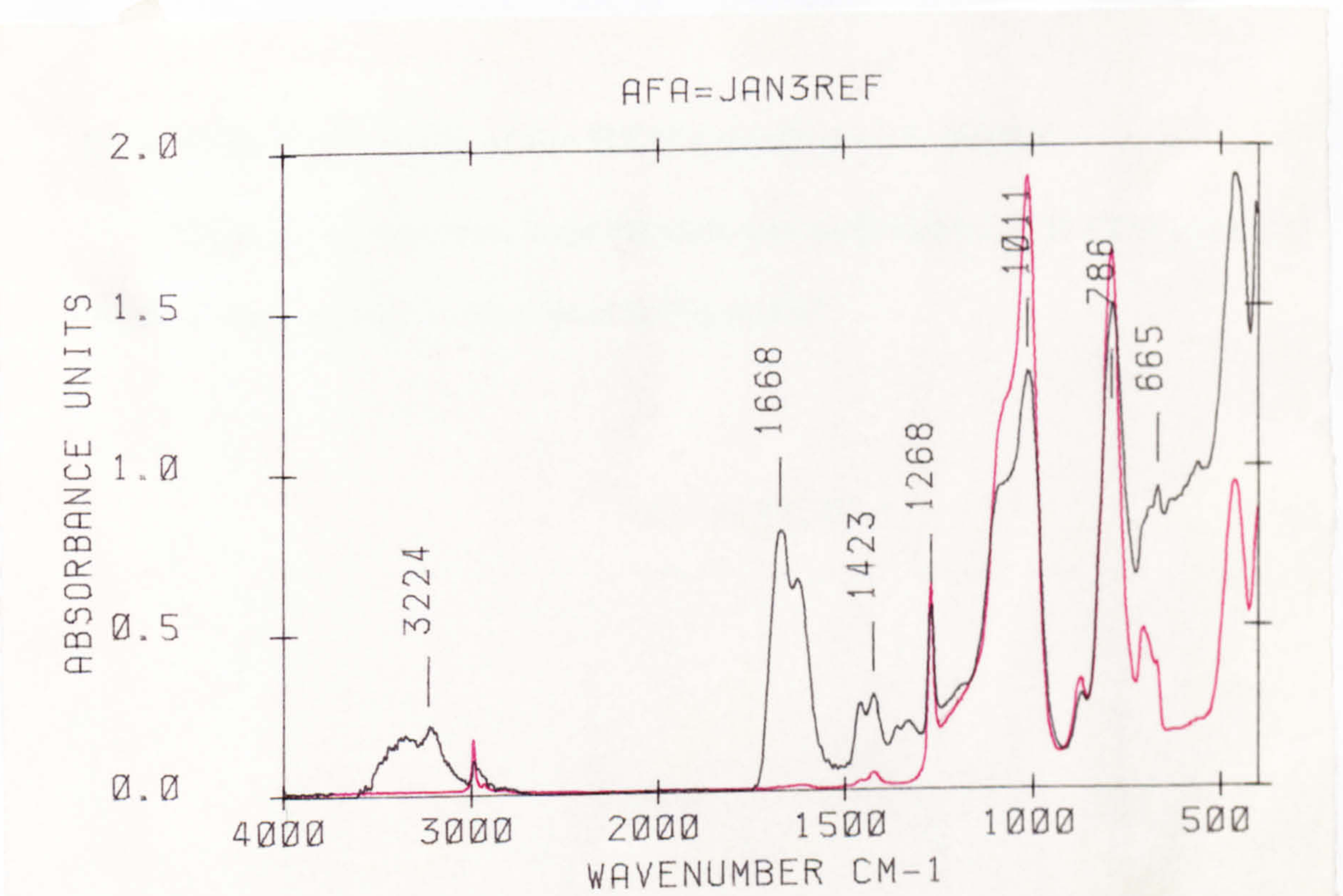


Figure 4.14 (a) ATR-FTIR spectra of acrylamide grafted onto the 1 pulse laser treated PDMS at 20 % wt concentration of AAm solution; (b) unmodified PDMS.

The characteristic absorption bonds of PAAm ($-CH_2-\underset{\underset{O}{\|}}{C}-C-NH_2$) appearing at 1609 - 1673 cm^{-1} and 3220-3400 correspond to the grafted carbonyl and amine groups of AAm

respectively (Figure 4.14a). These groups did not appear in the spectrum of the unmodified sample (Figure 4.14b). Comparison of these spectra with that of the unmodified one gives evidence for the presence of grafted poly AAm onto the modified surfaces of PDMS.

4.2.4 ATR-FTIR study of the HEMA grafted onto PDMS

The presence of surface bond PHEMA was confirmed by ATR-FTIR spectra of PDMS surfaces grafted as illustrated in Figure 4.15.

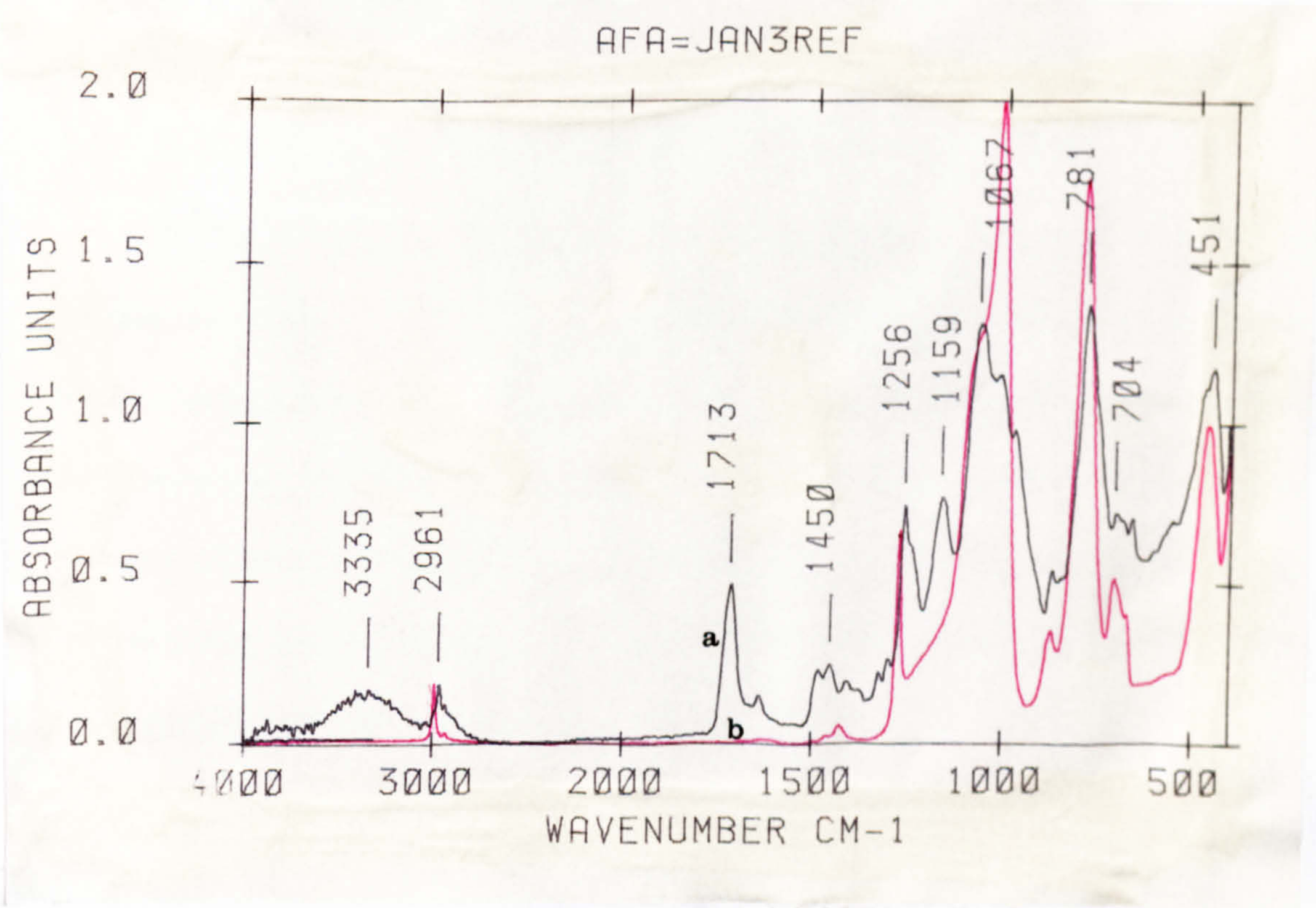


Figure 4.15 (a) ATR - FTIR spectra of HEMA grafted onto the 1 pulse laser treated PDMS at 20 % wt concentration of HEMA solution; (b) untreated PDMS.

The characteristic absorption bonds of PHEMA appearing at 1713-1718 cm^{-1} and 3335 cm^{-1} correspond to the grafted carbonyl and hydroxyl groups of PHEMA (Figure 4.15a). Also absorbance of characteristic peaks of PDMS such as (-Si-O-) and (Si-C) bond in the grafted PDMS decrease compared to the unmodified PDMS. Comparison of these spectra with that of the unmodified one (Figure 4.15b) gives evidence for the presence of grafted poly HEMA onto the modified surfaces of PDMS. The graft density can be also controlled by varying the pulse number and concentration of HEMA solution. A concentration of HEMA in the range of 10 - 30 wt % were used in the experiments. As shown, a maximum graft density was obtained using one pulse laser beam and 30 wt % HEMA solution.

4.2.5 ATR-FTIR study of the HEMAPC grafted onto PDMS

Figure 4.16 shows the ATR-FTIR spectra of PHEMAPC grafted onto the PDMS surface. According to the formula of HEMAPC (Table 1) characteristic absorbance bonds which appear at 3337 cm^{-1}, 1717 cm^{-1} and 1481 cm^{-1} contribute to the $-N^{+}Me_3$ and carboxyl groups of HEMAPC and also absorbance of PDMS bonds were decreased. Hence ATR-FTIR spectra indicate grafting of acrylate monomer onto the PDMS surface which have previously been irradiated by laser. Concentrations of HEMAPC in the range of 1-5 wt% were used in these experiments.

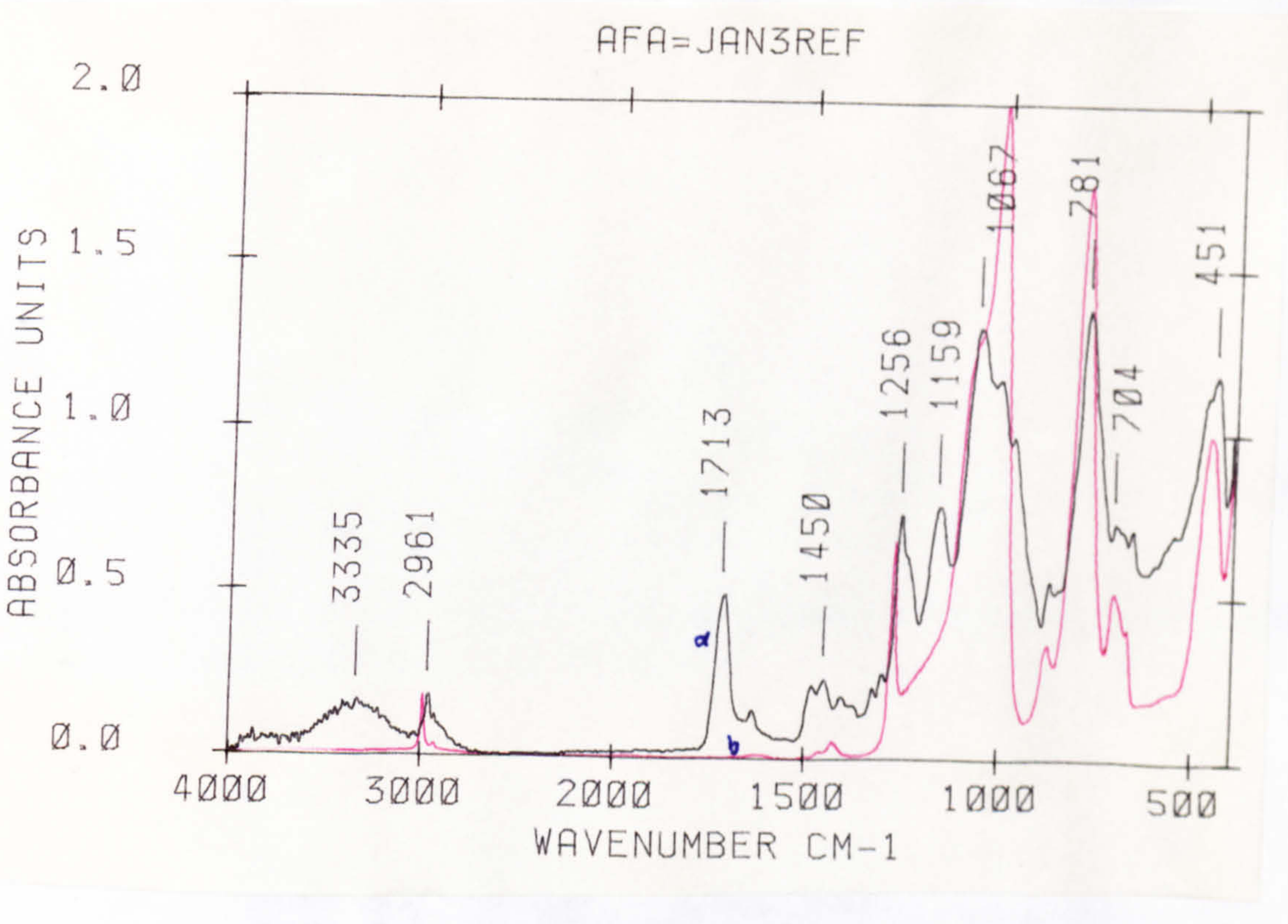


Figure 4.16 (a) ATR - FTIR spectra of HEMAPC grafted onto the 1 pulse laser treated PDMS at 2 % wt concentration of AAm solution; (b) untreated PDMS.

4.2.6 EDXA study of the acrylate grafted onto PDMS

Figures 4.17 and 4.18 show the EDXA analysis for AAm and HEMA grafted onto the PDMS surface. EDXA analysis show a higher percentage of nitrogen/oxygen (attributed to the grafted PAAm) on the surface of the sample modified with AAm

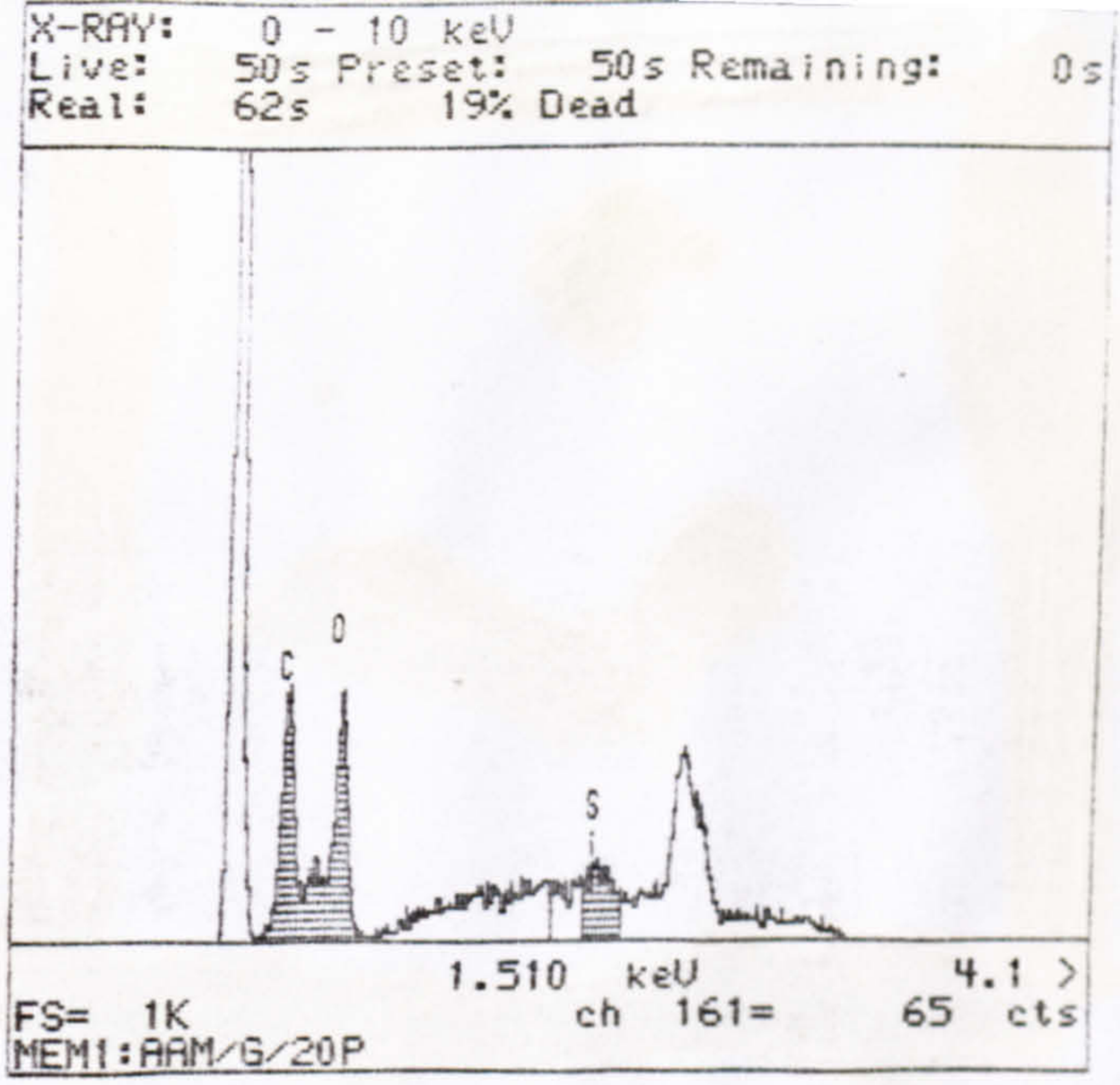


Figure 4.17 EDXA analysis of the acrylamide grafted onto the 5 pulses laser treated PDMS at a wavelength of 9.58 μm (1043 cm^{-1}).

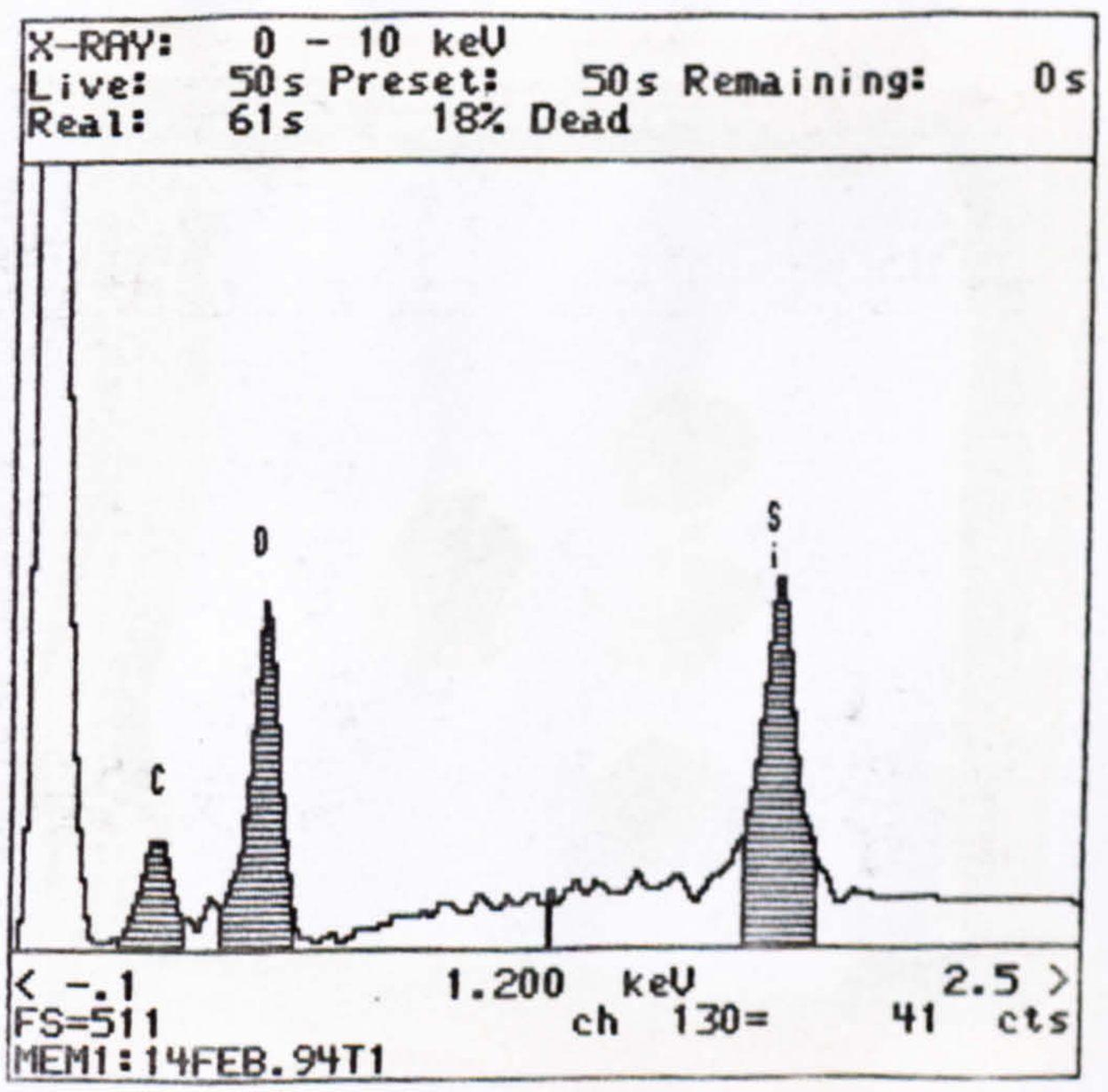


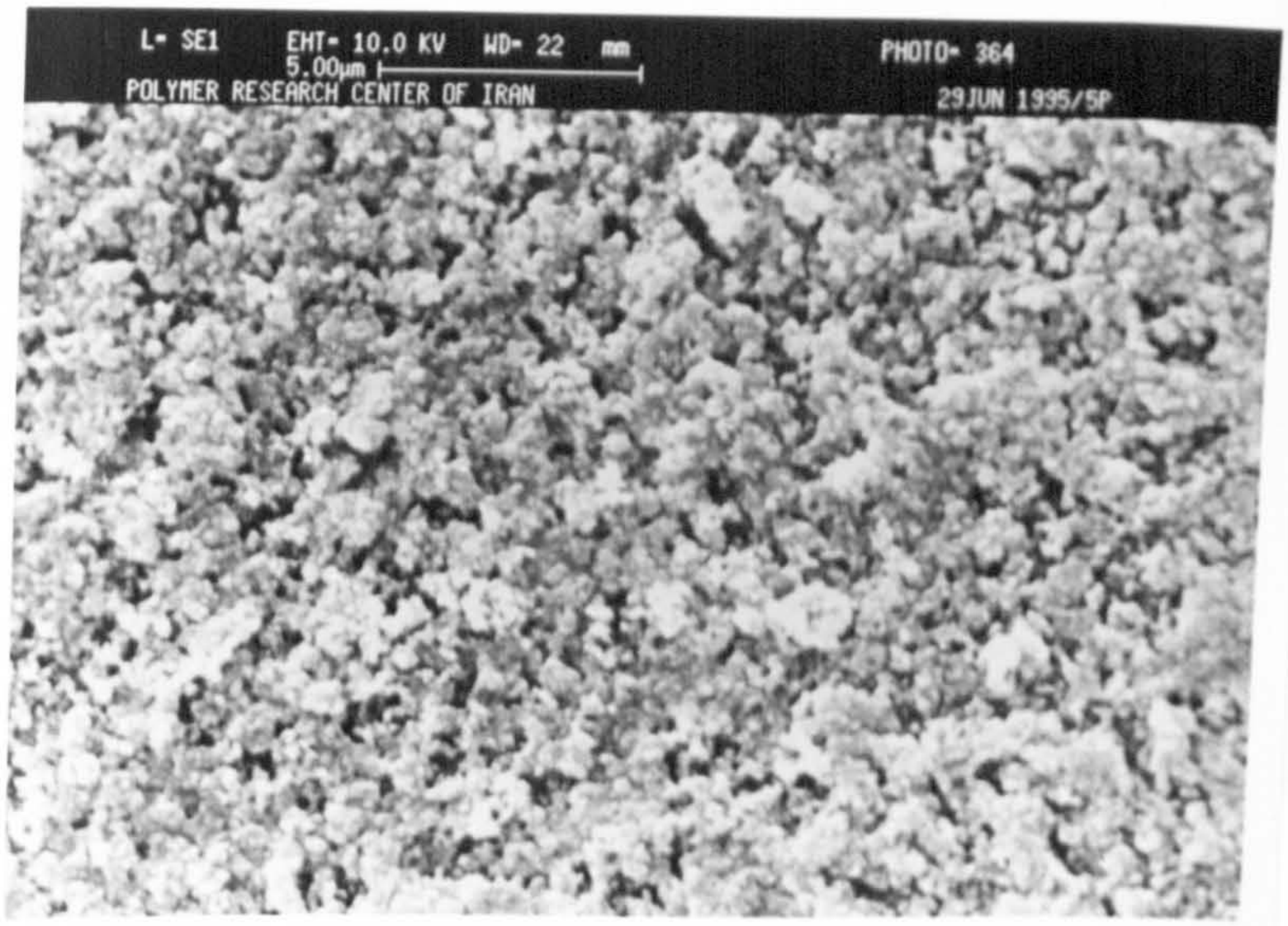
Figure 4.18 EDXA analysis of the HEMA grafted onto the 5 pulses laser treated PDMS at a wavelength of 9.58 μm (1043 cm^{-1}).

(36% C, 41% O, 15% N). Also EDXA analysis shows a higher percentage of carbon on the surface of HEMA-grafted silicone rubber film (42% C, 24% O, 32% Si) compared to the unmodified and the laser treated samples and almost resembles the EDXA spectrum of PHEMA. This is consistent with the ATR-FTIR spectrum of an PDMS sample modified with AAm and HEMA and show the graft layer of these polymers onto the PDMS surface.

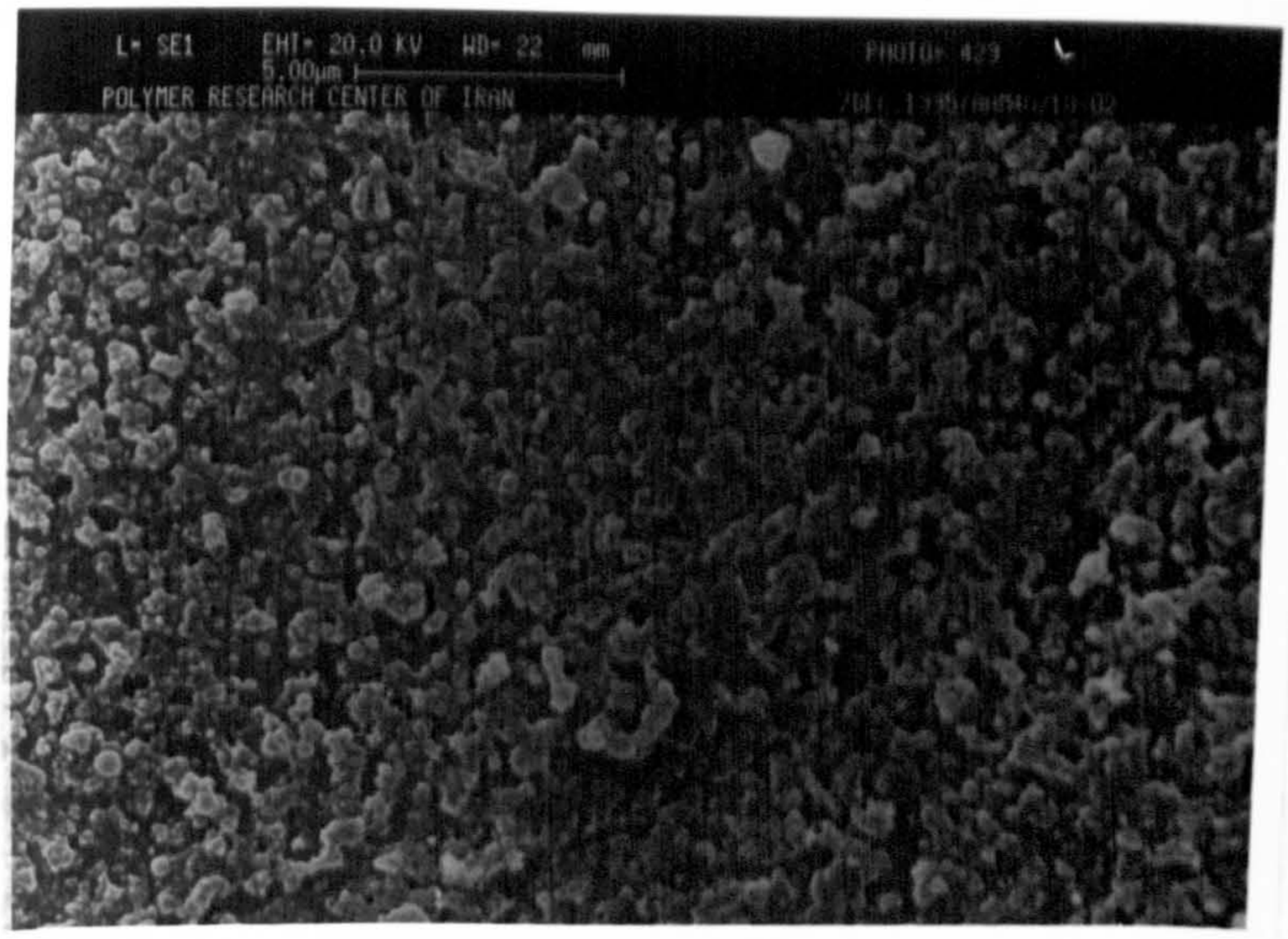
4.2.7 Morphology study of the AAm grafted onto PDMS

Respective SEM micrographs of the PDMS control, laser treated and polyacrylamide modified PDMS rubber films are presented in Figure 4.19.

Figure 4.19 (a) SEM micrograph (magnification is 5000 x) of control sample.



(b)



(c)

Figure 4.19 SEM micrograph (magnification is 5000 x) of: (b) laser treated PDMS by 5 pulses at 9.58 μm (1043 cm^{-1}); (c) acrylamide grafted PDMS which was pre-treated by 5 laser pulses beam at 9.58 μm (1043 cm^{-1}) wavelength and AAm solution of 20 wt%.

Comparison between the control (Figure 4.19a), laser treated (Figure 4.19b) and AAm modified films (Figure 4.19c) show that the surface of the AAm modified silicone rubber and CO_2-laser treated are rough but homogeneous. Control samples have a smooth surface without any contrast, and after treatment with laser, a continuous and homogenous porosity on the surface; the surfaces grafted with AAm show a rough surface.

Phase-contrast optical photomicrographs and SEM micrographs of the control sample and a treated sample on which the grafting process was carried out and then surface stained with eosin and an *untreated* sample on which the grafting process was carried out, are shown in Figures 4.20(a,b) and 4.21 respectively.

Figure 4.20 **Phase contrast micrographs of; (a) untreated PDMS without grafting process and stained with eosin.**

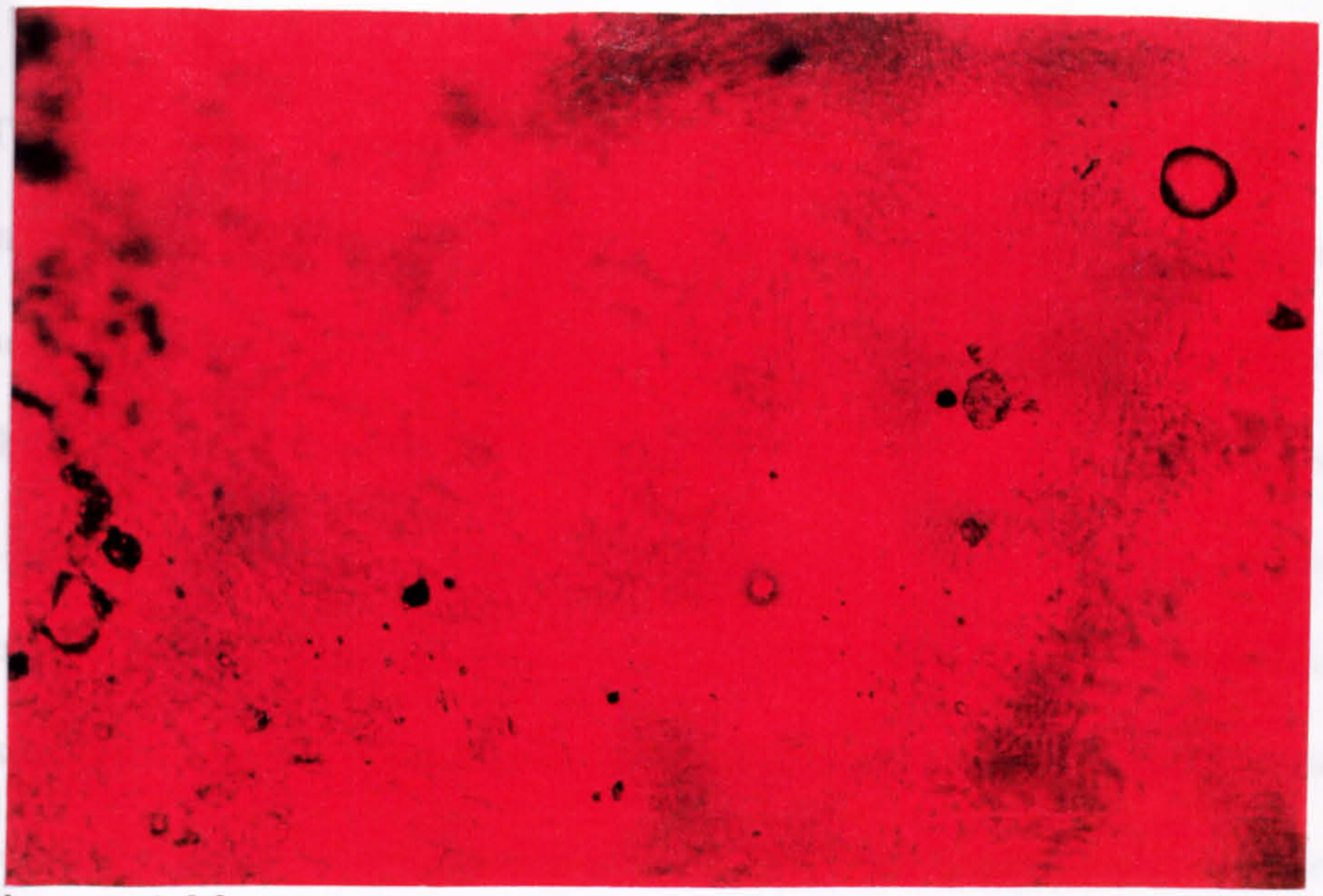
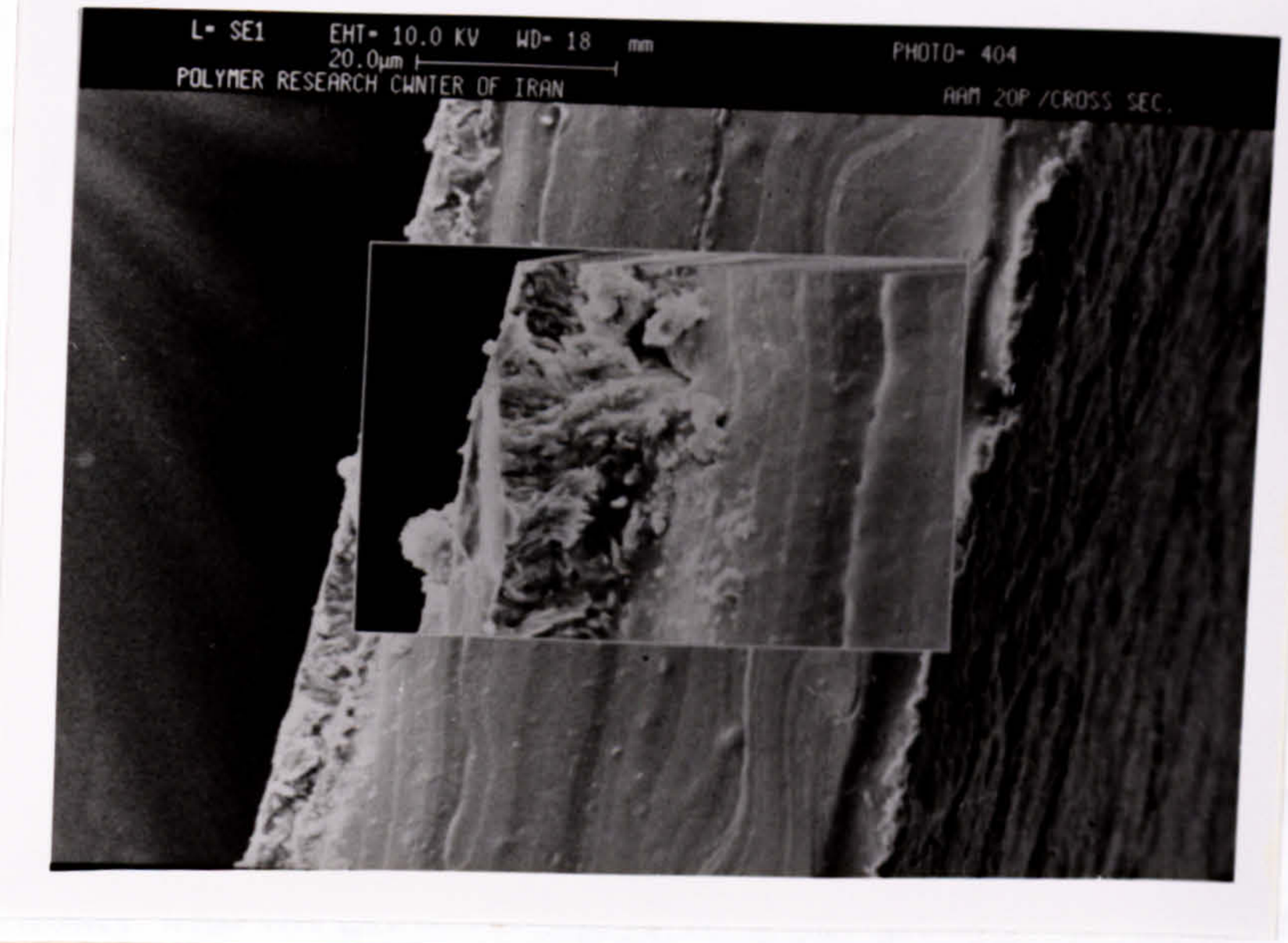


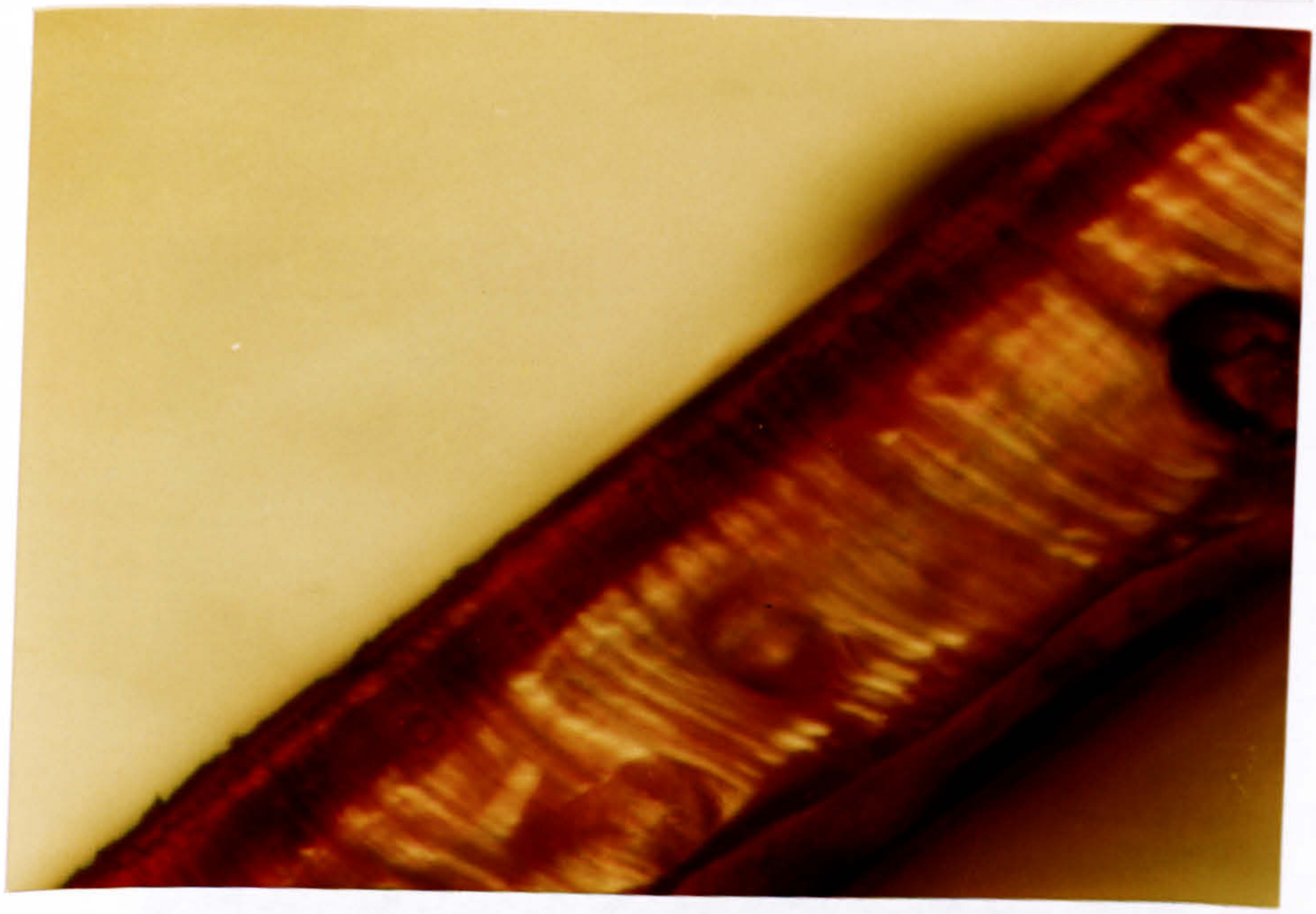
Figure 4.20 **Phase contrast micrographs of: (b) treated PDMS at 9.58 μm (1043 cm^{-1}) wavelength with 5 laser pulses and surface grafted using 20 wt % AAm monomer concentration solution and stained with eosin.**

Figure 4.21 **SEM micrograph (magnification is 5000 x) of untreated PDMS on which the grafting process was carried out.**

The fine layer of graft at the surface is seen to alter considerably the surface morphology. Figure 4.20a shows the control sample which was not stained by eosin, because no grafted layer of AAm is present on this surface and eosin only stains the characteristic groups such as carboxyl and hydroxyl and amine groups. But Figure 4.20b (treated and AAm grafted PDMS) showed a surface which is stained red by the eosin. Figure 4.21, in which untreated PDMS has been put through this process, as expected, does not show any graft layer of PAAm. These results indicate that only samples treated by the laser and which contain peroxide groups are capable of initiating graft polymerization onto the surface of the PDMS. Figure 4.22(a,b) shows the SEM and optical micrographs of the cross section of an AAm grafted PDMS film with a graft density of 22×10^{-4} g cm^{-2} after staining with eosin. The thickness of the grafted layer is estimated from this photograph to be approximately 20 μm; as can be seen clearly, the graft polymerization is restricted to the surface region of the film, indicating that only the surface region was modified without altering the bulk properties. As shown in this photograph, both sides of film were subjected to graft polymerization. When only the one side of the film was irradiated, the irradiated side was grafted and stained. It is evident that laser irradiation only produces peroxides in the exposed surface region of the film. It is also highly probable that the graft monomer penetration is limited to the surface region, because the degree of graft polymerization was accelerated with an increase in monomer concentration.



(a)



(b)

Figure 4.22 (a) SEM micrograph of cross section of an acrylamide grafted PDMS film which was pre-treated by 1 laser pulse beam at 9.58 µm (1043 cm^{-1}) wavelength and AAm solution of 20 wt % ; (b) optical micrograph of the cross section of an AAm grafted PDMS film after staining with eosin.

4.2.8 Morphology of the HEMA and HEMAPC grafted onto the PDMS

SEM micrographs of the HEMA and HEMAPC grafted silicone rubber films are represented in Figure 4.23(a,b). Comparison between control (Figure 4.19a), HEMA modified films (Figure 4.23a) and HEMAPC (Figure 4.23b) show that the rough surface of a PHEMA and HEMAPC modified silicone rubber are homogeneous, like the AAm grafted silicone rubber. Control samples have a smooth surface without any contrast, and after laser treatment, continuous and homogenous porosity onto the surface was formed, whilst the grafted surfaces with PHEMA and HEMAPC show a rough surface. Figure 4.24 shows the typical SEM micrograph of HEMA modified silicone rubber with the graft density of 6.5×10^{-3} g/cm^2. As can be seen, the graft density is very high and the overall morphology of this surface is smooth.

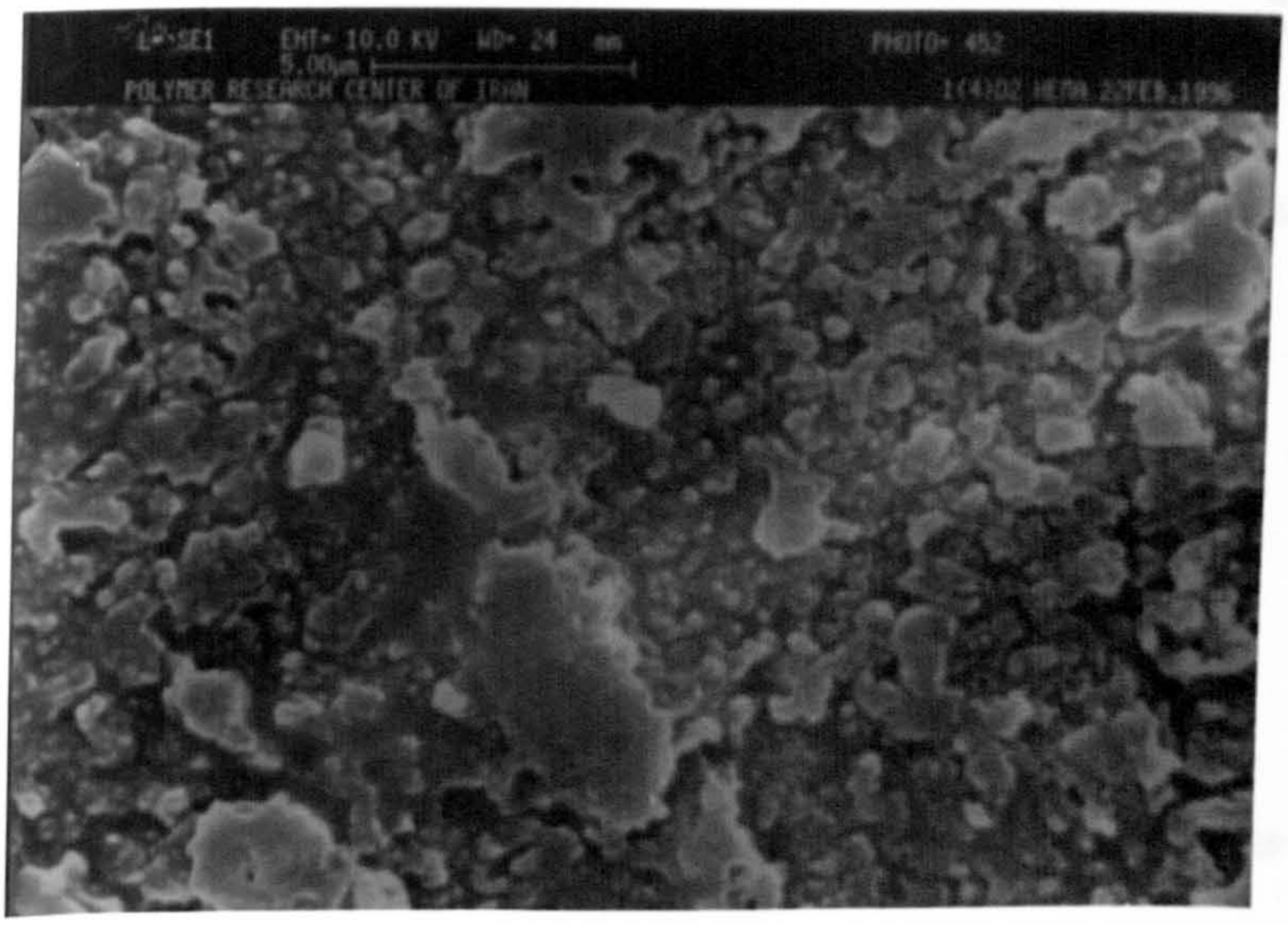


Figure 4.23 (a) SEM micrograph (magnification is 5000 x) of HEMA grafted PDMS which was pre-treated by 5 laser pulses beam at 9.58 μm (1043 cm^{-1}) wavelength and HEMA solution of 20 wt %.

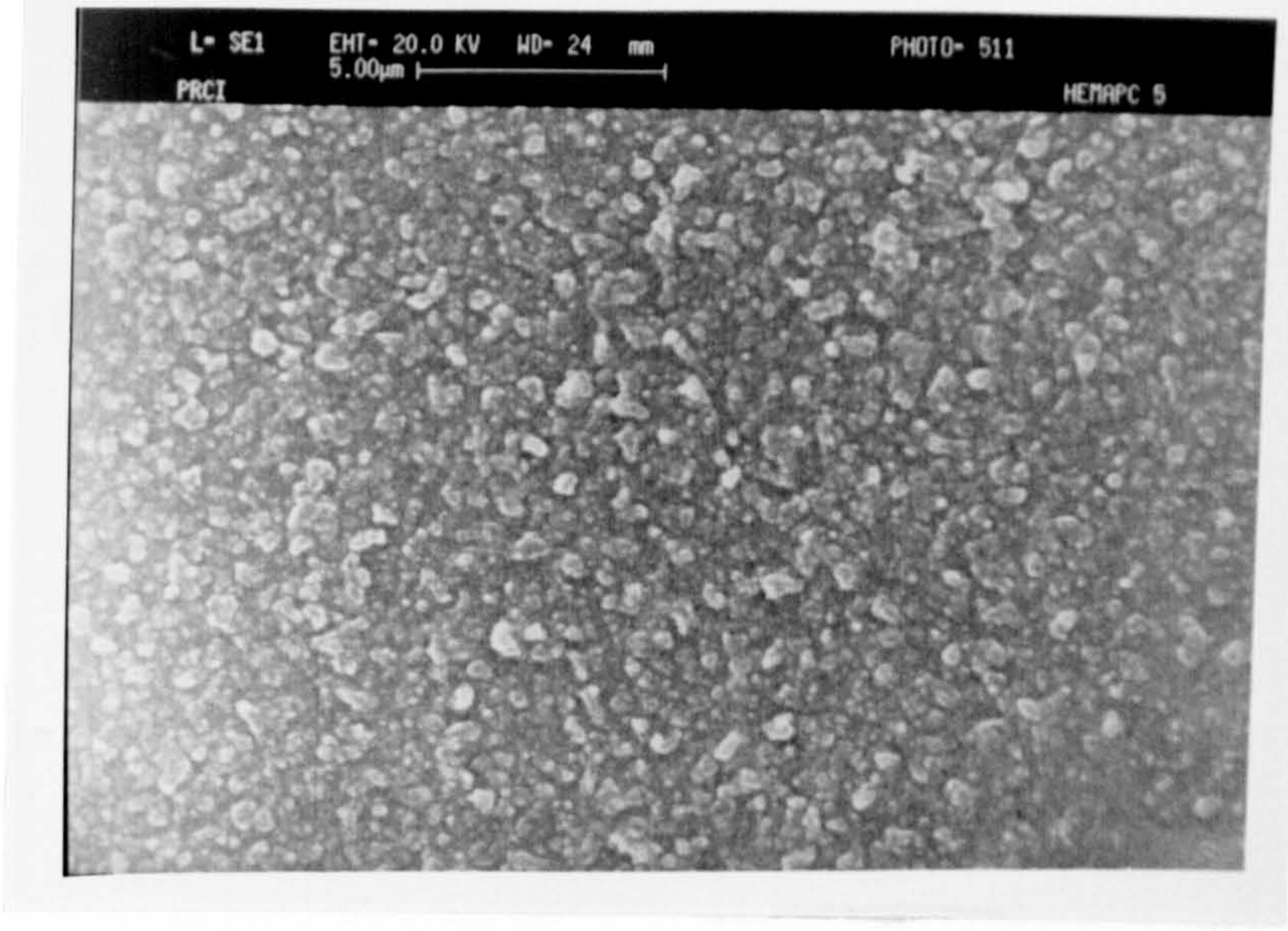


Figure 4.23 (b) SEM micrograph (magnification is 5000 x) of HEMAPC grafted PDMS which was pre-treated by 5 laser pulses beam at 9.58 μm (1043 cm^{-1}) wavelength and HEMAPC solution of 3 wt %.

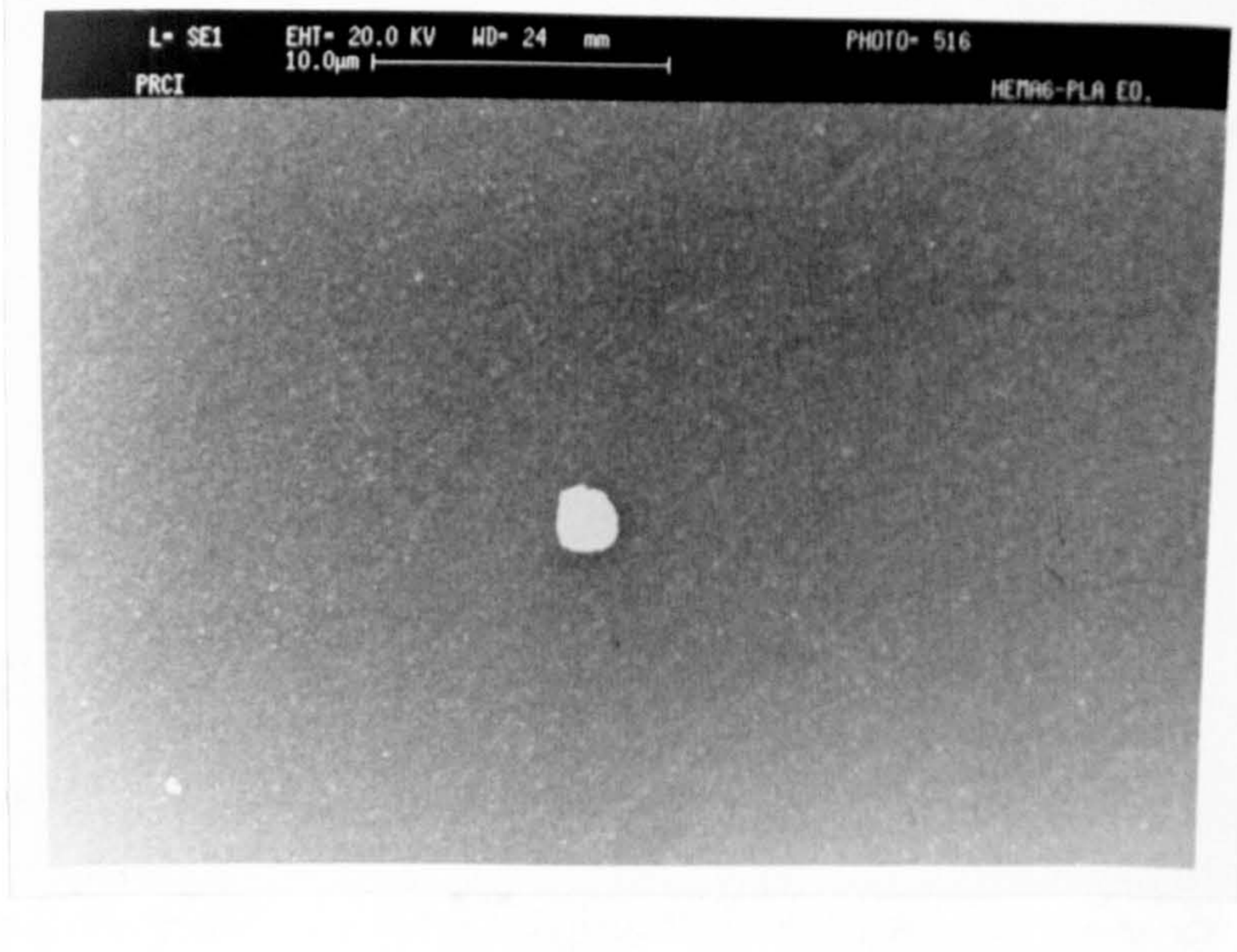


Figure 4.24 SEM micrograph (magnification is 4000 x) of HEMA grafted PDMS which was pre-treated by 1 laser pulse beam at 9.58 μm (1043 cm^{-1}) wavelength and HEMA solution of 30 wt %; graft density is 6.5×10^{-3} g cm^{-2}.

This result indicates that by increasing the thickness of the graft layer onto the silicone rubber surface, both treated surfaces, which are rough and covered with pores, change to a smooth, relatively non-porous surface. These two different morphologies have a significant effect on blood compatibility, as will discussed in section (4.3.7).

SEM and optical micrographs of cross sections are the same as cross sections of AAm modified silicone rubber, which have been shown in Figure 4.22.

4.2.9 Effect of pulse number on graft density

The effect of pulse number on the graft polymerization of AAm onto the PDMS films is shown in Table 4.3. Higher graft densities are obtained on the PDMS film with fewer pulse numbers. The differences are understandable from the results of peroxide formation described in section (4.2.1). Thus, it is not necessary to treat the PDMS film with a high number of pulses, since even a single pulse is very effective in introducing polymeric peroxides onto the PDMS film surface, as demonstrated above. As required, the graft density can be controlled by varying the pulse number and the concentration of acrylate solution. Concentrations of AAm and HEMA in the range of 10 - 30 wt % were used in our experiments. Figure 4.25 shows the ATR-FTIR spectra of AAm grafted onto silicone rubber at 2 different concentration of AAm solution. The maximum graft density was obtained at a single pulse laser irradiation and 30 wt % AAm solution. However the same results were obtained for HEMA and HEMAPC grafted onto the PDMS surfaces, but for HEMAPC the monomer concentration was kept low, because, by increasing the monomer concentration, the solution converted to a gel (homopolymer formation rather than the grafted PHEMAPC) and the surface

Table 4.3 Concentration of PAAm grafted onto the silicone surface pre-treated with CO_2- pulsed laser as a function of the pulse number of laser beam at 9.58 μm (1043 cm^{-1}) wavelength.

Pulse number	Grafted PAAm in g/cm^{-2}
1	20 x 10^{-4}
3	14 x 10^{-4}
5	10 x 10^{-4}
7	8 x 10^{-4}
10	7 x 10^{-4}
15	6 x 10^{-4}
20	4 x 10^{-4}

graft density decreased. In this case the best monomer concentration is about 1-5 wt % (Figure 4.26).

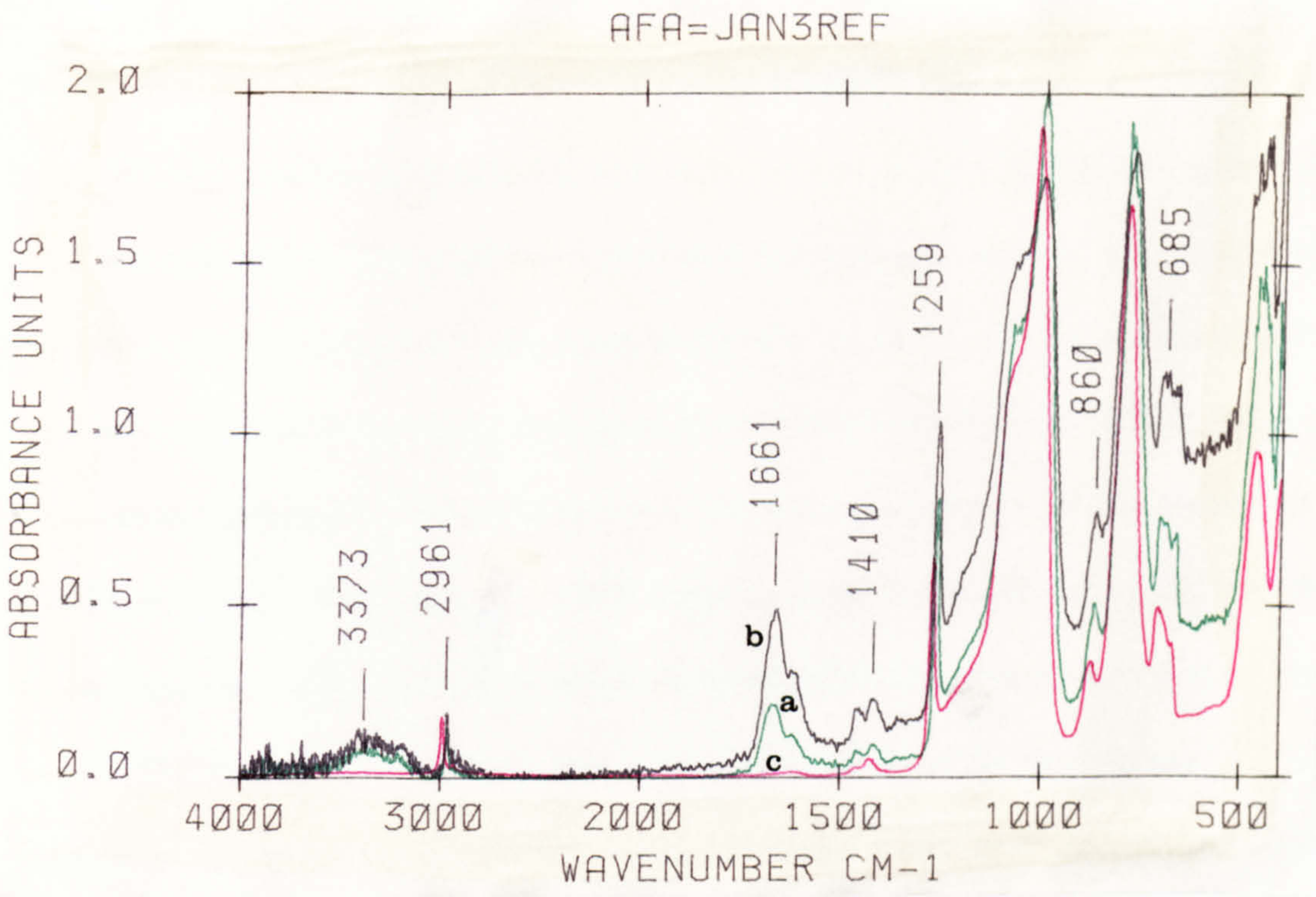


Figure 4.25 **ATR-FTIR spectra of pre-treated PDMS with 5 laser pulses at 9.58 μm (1043 cm^{-1}) wavelength grafted with: (a) 10 wt % AAm solution; (b) 20 wt % AAm solution; (c) unmodified PDMS.**

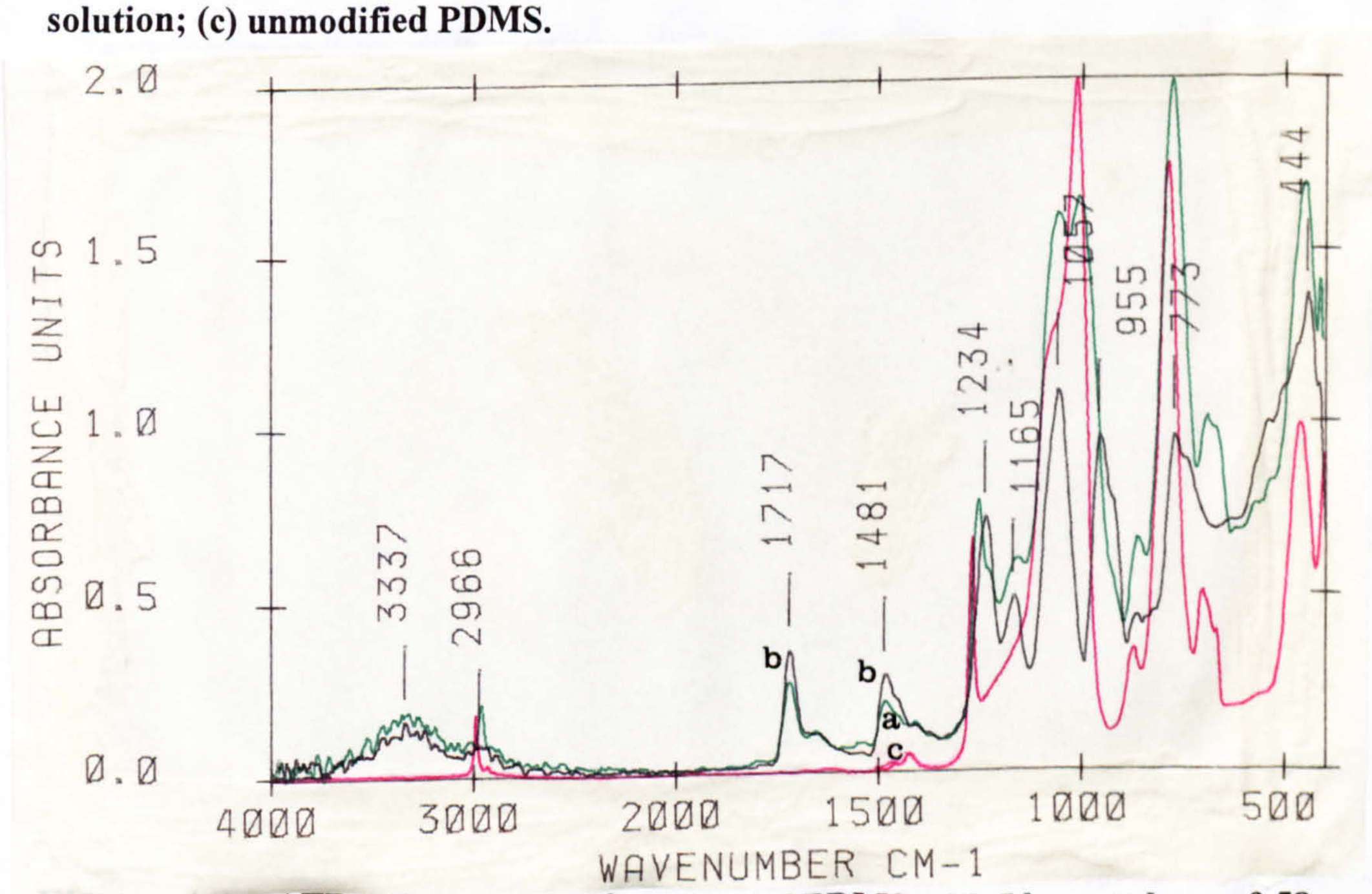


Figure 4.26 **ATR-FTIR spectra of pre-treated PDMS with 5 laser pulses at 9.58 μm (1043 cm^{-1}) wavelength with: (a) 1 wt % HEMAPC solution; (b) 3 wt % HEMAPC solution; (c) unmodified PDMS.**

4.2.10 Wettability study of the AAm grafted onto PDMS

PDMS exhibits hydrophobic behaviour and poor wettability. The surface of PDMS film becomes hydrophilic upon graft polymerization with AAm. The water drop contact angle changes as the pulse number is increased with the increasing of pulse number up to 5 pulses. It means that the surface of treated samples have been changed and a completely hydrophobic surface was obtained compared with the unmodified PDMS. The virgin PDMS film has a 105° water drop contact angle. In comparison, the single pulse laser treated sample, without any graft polymerization gave a contact angle of 170°. The measurement of the water drop contact angle of the single-pulse, grafted surface in the presence of 20 wt% AAm solution, shows that the contact angle decreased to 30° and a hydrophilic surface was obtained (Figure 4.27).

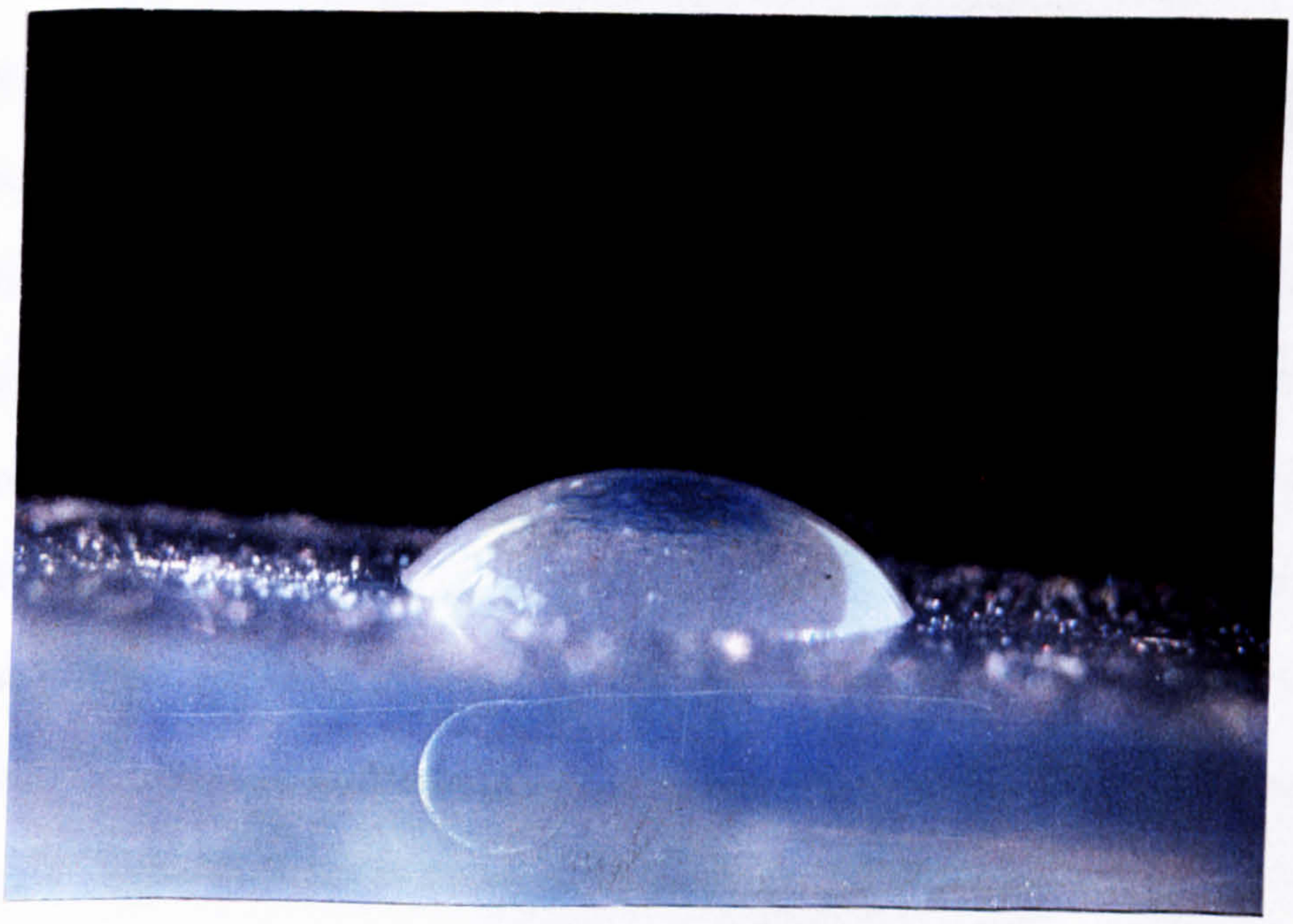


Figure 4.27 Water drop contact angle of the AAm grafted onto the PDMS surface which was pre-treated by laser beam at 9.58 μm (1043 cm^{-1}) and 20 % wt concentration solution of AAm.

Table 4.4 **Comparison of water drop contact angle for PDMS samples.**

Sample	Water drop contact angle (degree)
Untreated PDMS (control)	105
Treated PDMS by single laser pulse at 9.58 μm wavelength	170
Grafted PDMS pre-treated by single laser pulse at 9.58 μm wavelength, with AAm	35

The contact angle values of the ungrafted and the grafted samples are listed in Table 4.4.

Graft copolymerization of a water-soluble monomer (AAm) produces a permanently hydrophilic surface onto the surface of hydrophobic polymeric substrates which is entirely different from that produced upon simple oxidation by the CO_2-pulsed laser. This is because the surface modified by the graft copolymerization carries many macro-molecular chains which are covalently attached to the polymer substrate.

Our results show the potential of using the CO_2-pulsed laser to induce grafting of acrylamide onto the surface of PDMS by a pre-irradiation method, without photosensitizer, when the wavelength of the laser beam corresponds to the infrared absorption of the PDMS.

The surface of the chemically inert PDMS can be graft polymerized with water-soluble acrylamide monomer when pre-treated with a CO_2-pulsed laser in the oxygen atmosphere and then thoroughly degassed as mentioned in section (4.2.2).

4.2.11 Wettability study of the HEMA and HEMAPC grafted onto PDMS

The surface of PDMS film has became hydrophilic upon graft polymerization of HEMA and HEMAPC monomers. After treatment of PDMS by CO_2-pulse laser, the water drop contact angle changes as the pulse number is increased. The measurement of the water drop contact angle of the laser grafted surface with a single pulse and 30 wt% HEMA solution, shows that the contact angle decreased to 25° and a hydrophilic surface was obtained. Measurement of the contact angle of water on a sample surface is used to provide an estimation of the hydrophilicity of the surface[2]; the lower the contact angle, the higher the hydrophilicity of the modified surface. Results show that there are differences between the hydrophilicity of AAm, HEMA and HEMAPC treated surfaces. The water drop contact angle of HEMAPC grafted onto the PDMS surface by a single laser pulse and 5 wt % monomer concentration is about 10°. These differences are attributed to the chemical structure of the different acrylate polymers grafted onto the PDMS surface and as will be seen in the *in vitro* study (section 4.3.7), the degree of hydrophilisity has a major effect on blood compatibility.

4.3 *In Vitro* Study

This study was undertaken to examine the effect of treated polymer surfaces on platelet adhesion. The need is for a reduction or limitation of platelet adhesion because

interactions of these blood components with man-made materials will-trigger thrombus formation on the foreign surface when it comes into contact with blood. The rationale for graft polymerization of water-soluble monomers on polymer surfaces to reduce platelet adhesion is based on the minimal protein interaction of certain hydrogels, which are widely employed in protein characterization and culture of anchor-independent cells. Among such hydrogels are the polyacrylate gels. The characteristic of these hydrogels is their very high water content, which is probably the main reason for their minimal protein adsorption and cell adhesion[135].

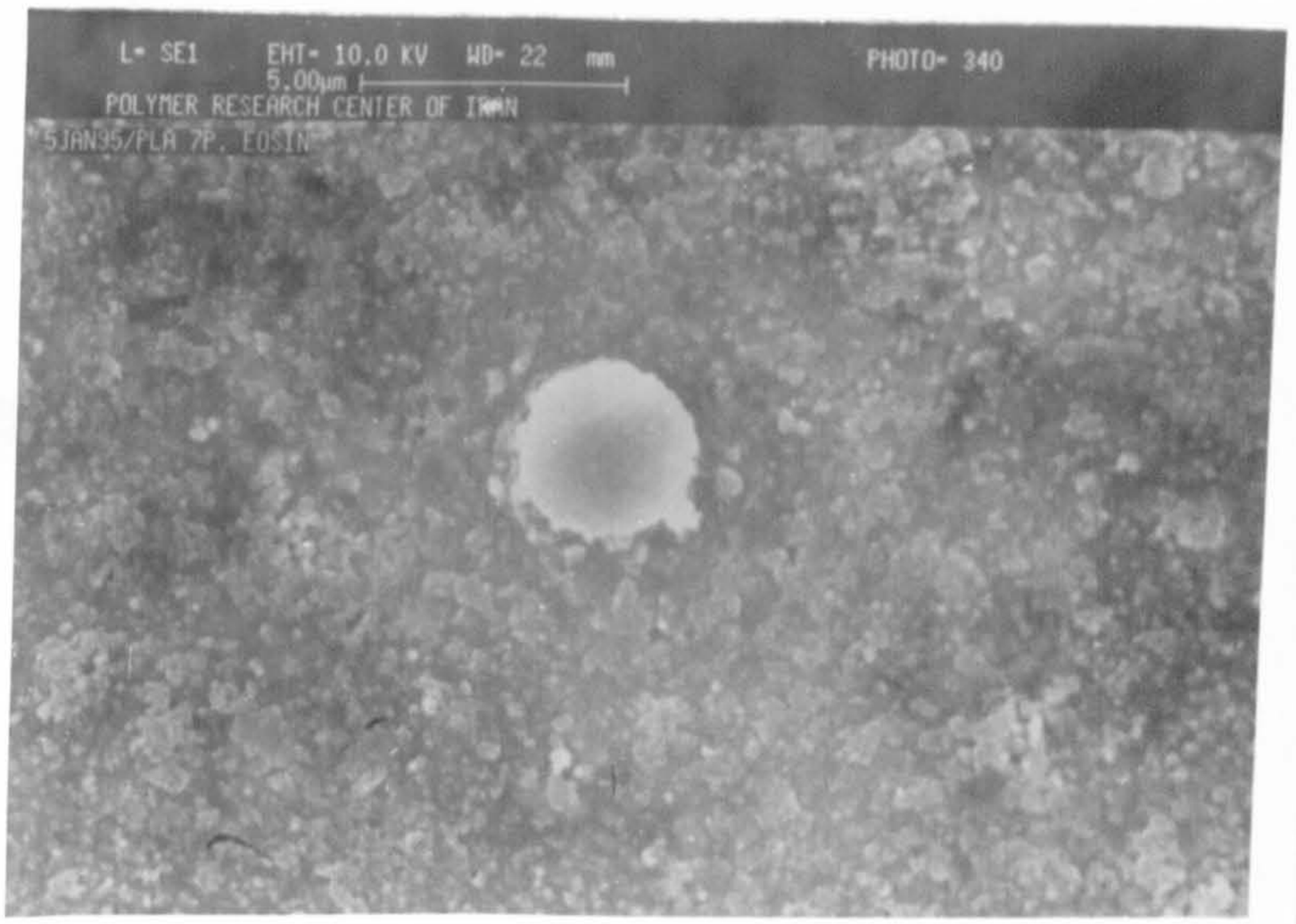
To determine the blood compatibility of the laser treated and acrylate grafted PDMS by laser, platelet adhesion studies were conducted.[210]

When a biologically incompatible material is in contact with blood, thrombus formation takes place on it via a rapid adsorption of plasma proteins and the subsequent adhesion of platelets. Numerous approaches to suppress the adhesion of blood components onto synthetic surfaces have been studied and both highly hydrophobic and hydrophilic surfaces are found to be useful.[208]

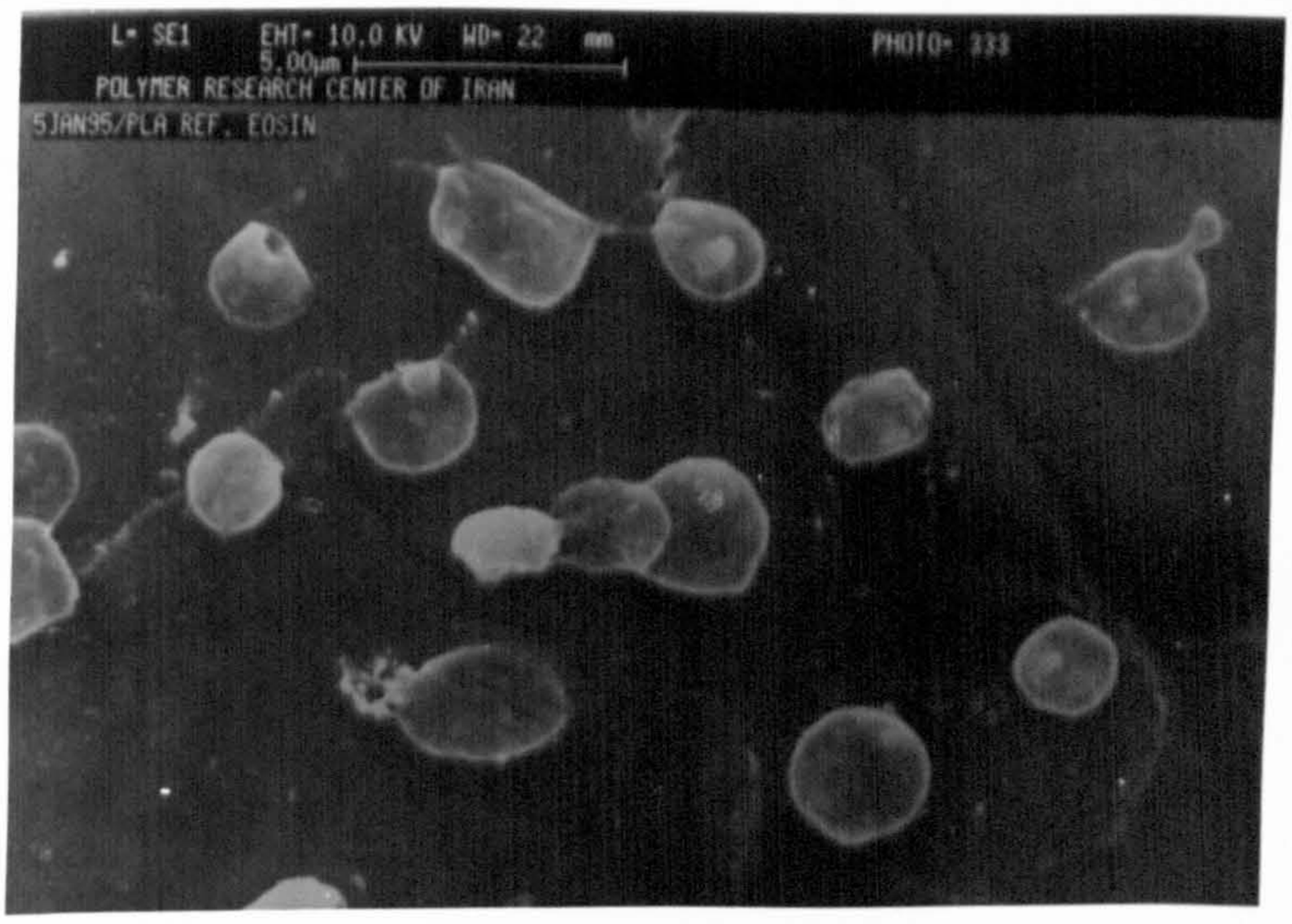
4.3.1 Platelet adhesion study onto the laser treated PDMS

Platelet adhesion studies were carried out *in vitro* using the platelet rich plasma (PRP) method.

SEM micrographs and photo light microscopy of the samples are shown in Figure 4.28(a,b) and 4.29(a,b,c) respectively.

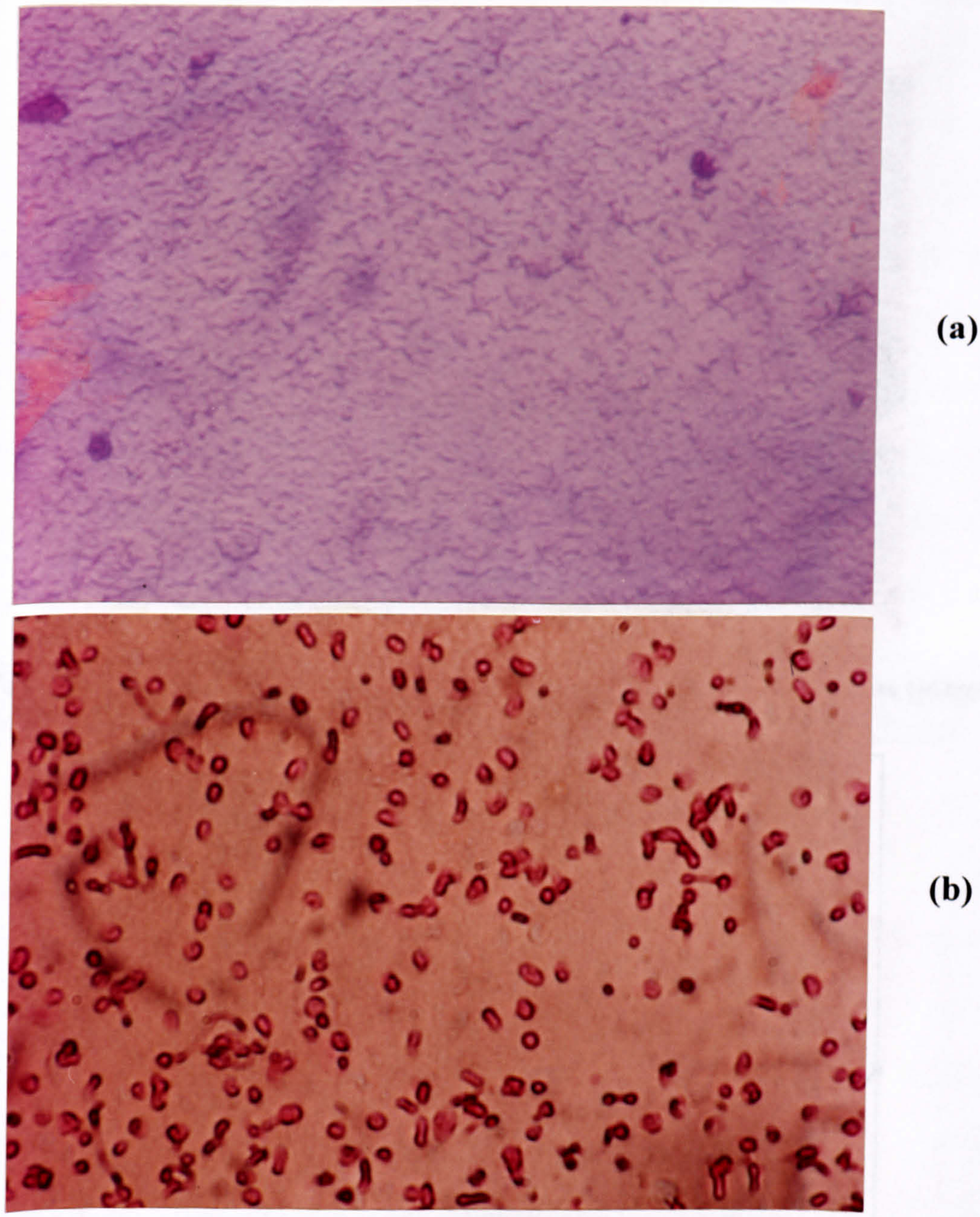


(a)



(b)

Figure 4.28 SEM micrograph (magnification is 5000 x) of : (a) adhered platelets onto PDMS treated with laser pulses beam by 10 pulses at 9.58 µm (1043 cm^{-1}) wavelength; (b) adhered platelets on the untreated PDMS surface.



(a)

(b)

Figure 4.29 **Optical photomicrographs of : (a) platelet attachment to treated PDMS at 9.58 μm (1043 cm^{-1}) wavelength (platelets were stained with eosin, and magnification is 1000 x); (b) platelet attachment to untreated PDMS (control))platelets were stained with eosin, and magnification is 1000 x).**

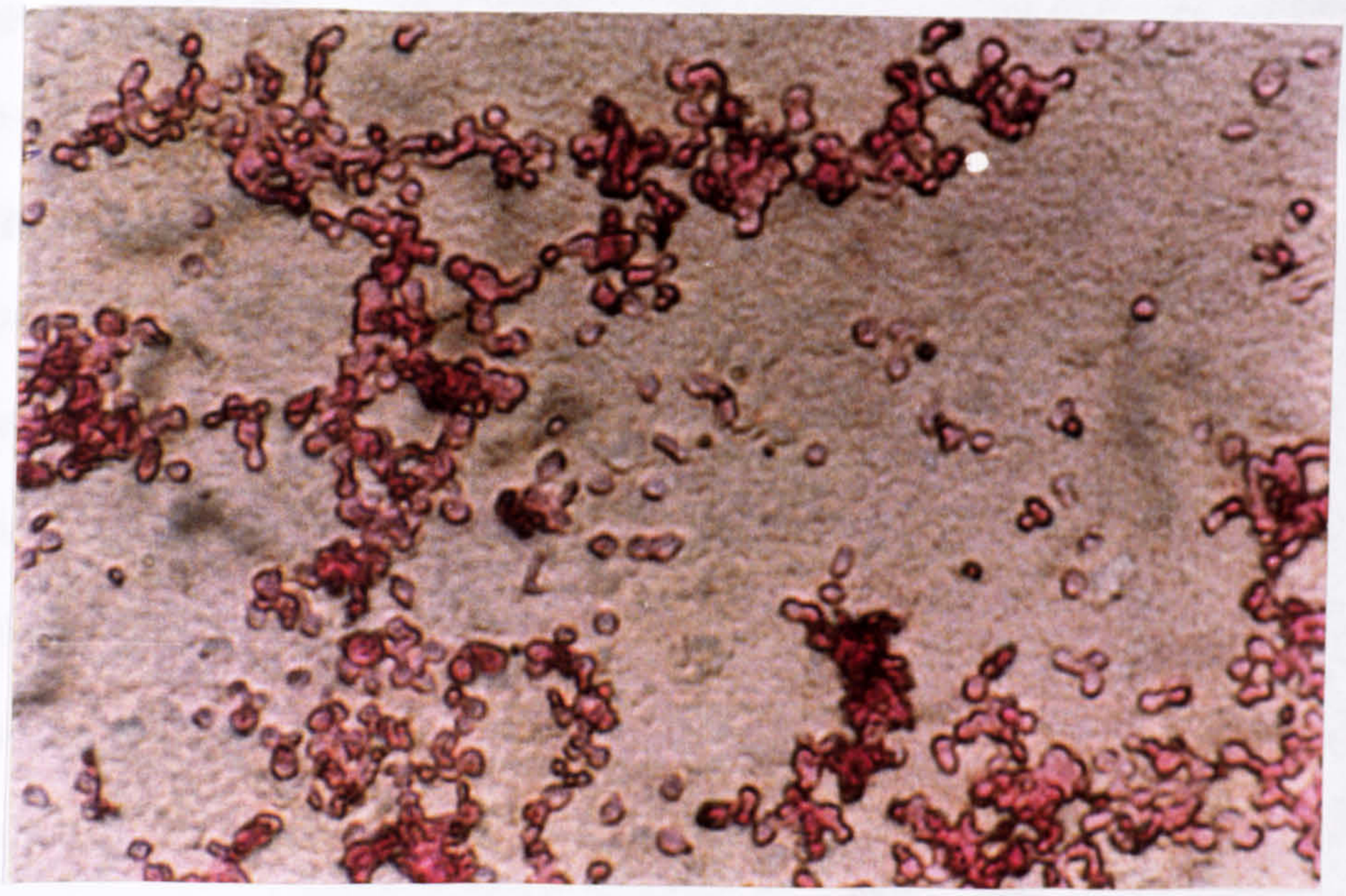


Figure 4.29 **(c) Optical photomicrograph of platelets attachment to glass (platelets were stained with eosin and magnification is 1000 x).**

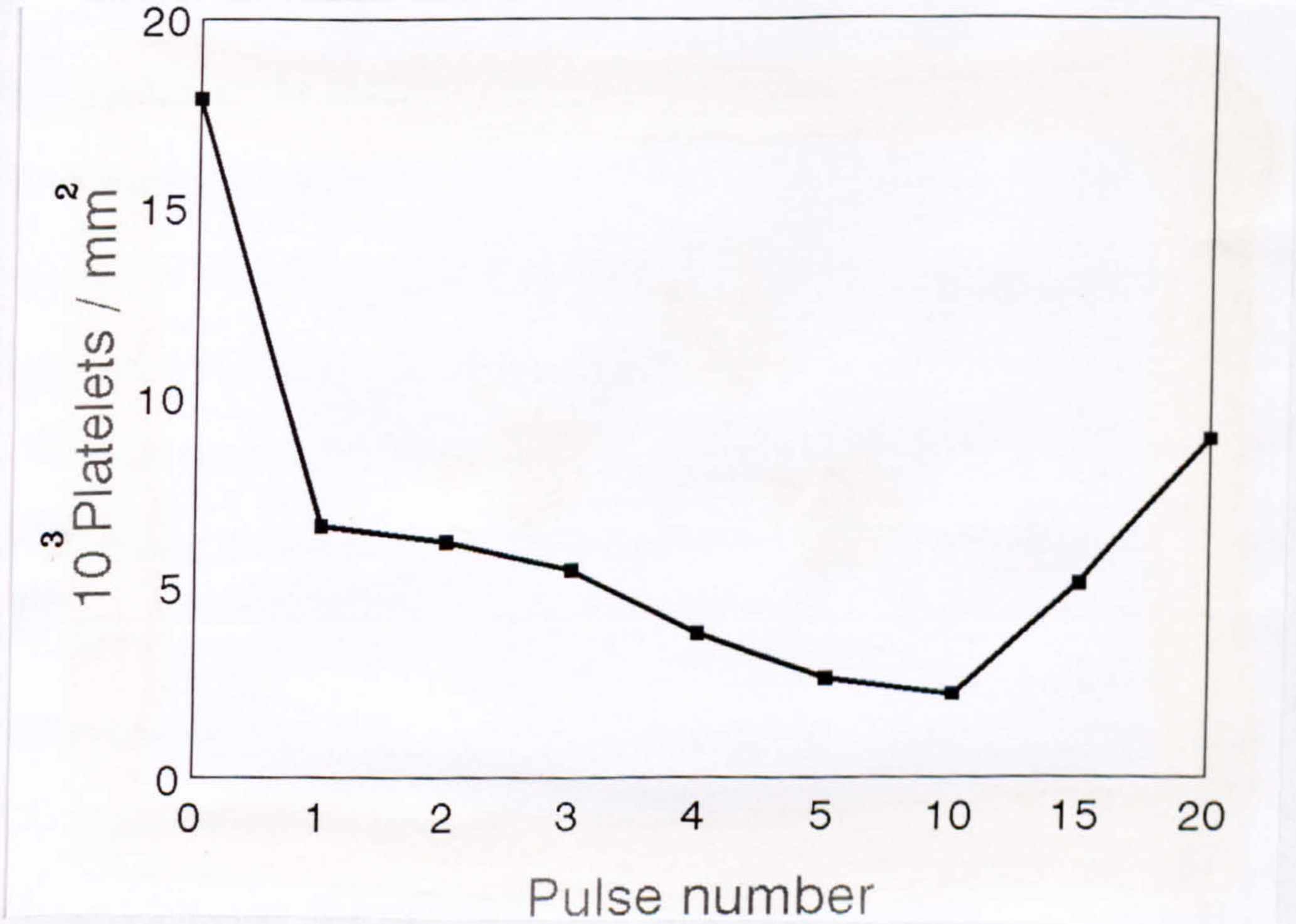


Figure 4.30 **Dependence of platelets adhesion on pulse number of the laser induced surface treated PDMS.**

As can be seen, no platelet spreading is observed on the laser treated PDMS in comparison with the unmodified sample and on glass, to which platelets adhered and became fully activated. On the untreated materials the platelets covered the surface completely and formed micro- thrombi. It can be concluded from these studies that the surface of treated PDMS does not induce platelet activation. The number of platelets adhered on the untreated and the treated PDMS films from PRP after different number of pulses was also obtained from the lactate dehydrogenase (LDH) method is shown in Figure 4.30. Platelet adhesion is at a maximum on the untreated PDMS while it is at a minimum on 10 pulses laser-treated samples. Exposure of a PDMS surface to the CO_2-pulsed laser for a very short time causes oxidation of the polymer but only in the surface region, regardless of the nature of the polymer. The decrease in platelets adhesion to the treated surfaces can be due to either the changed chemical nature of the surface or the changed morphology, or to a combination of both these changes. Chemically, we have demonstrated that the surface of the treated material has a number of peroxide, hydroxyl, carboxyl and carbonate groups, giving a high oxygen content, which might be altering the nature of recognition processes involving the surface at the platelets. However, it is evident that the nature of the surface is of major importance; the rough nature of the treated surfaces leave fewer suitable sites for the platelets to settle onto the surface. It has been reported that the porosity and super-hydrophobic have major effect on blood compatibility of the polymer surfaces[14,202,203]. Data from *in vitro* blood compatibility experiments indicated a significant reduction of platelet adhesion and aggregation for the modified surfaces and that those platelets, which were adherent, remained unspread. The extent of platelet adhesion was

correlated with the number of laser pulses.

There is a general agreement that *in vitro* tests are most useful in the evaluation of artificial surfaces that are highly reactive with blood. It is also agreed that human blood should be used for *in vitro* tests whenever possible. Evaluating hemocompatibility *in vitro* usually involves platelet assays and coagulation assays[195,211].

The rationale for surface modification of PDMS by laser treatment to reduce platelet adhesion is based on the minimal interaction of (the super-hydrophobic nature of) the PDMS laser-irradiated surfaces with blood components. It has been reported that the polymer surface, either super-hydrophilic or super-hydrophobic, which possess very high and very low surface free energies (γ_1) respectively, may possess excellent blood compatibility[14]. It is well known that thrombus formation is triggered by an interaction between blood components and the foreign polymer surface[14]. On the other hand, protein adsorption depends greatly upon the surface energy of the substrate[15]. As mentioned above such "hydrophobic" surfaces are widely employed to avoid blood coagulation and thrombus formation. The laser induced surface modification of polymers provides a unique and powerful method for the surface modification of polymeric materials without altering their bulk properties. This technique thus offers possibilities to improve the performance of existing biomaterials and medical devices and for developing new biomaterials[6,7].

4.3.2 Cell culture study onto the laser treated PDMS

Figures 4.31(a,b,c) and 4.32(a,b,c) show the attachment of Baby Hamster Kidney (BHK) cells onto PDMS treated and untreated films and glass.

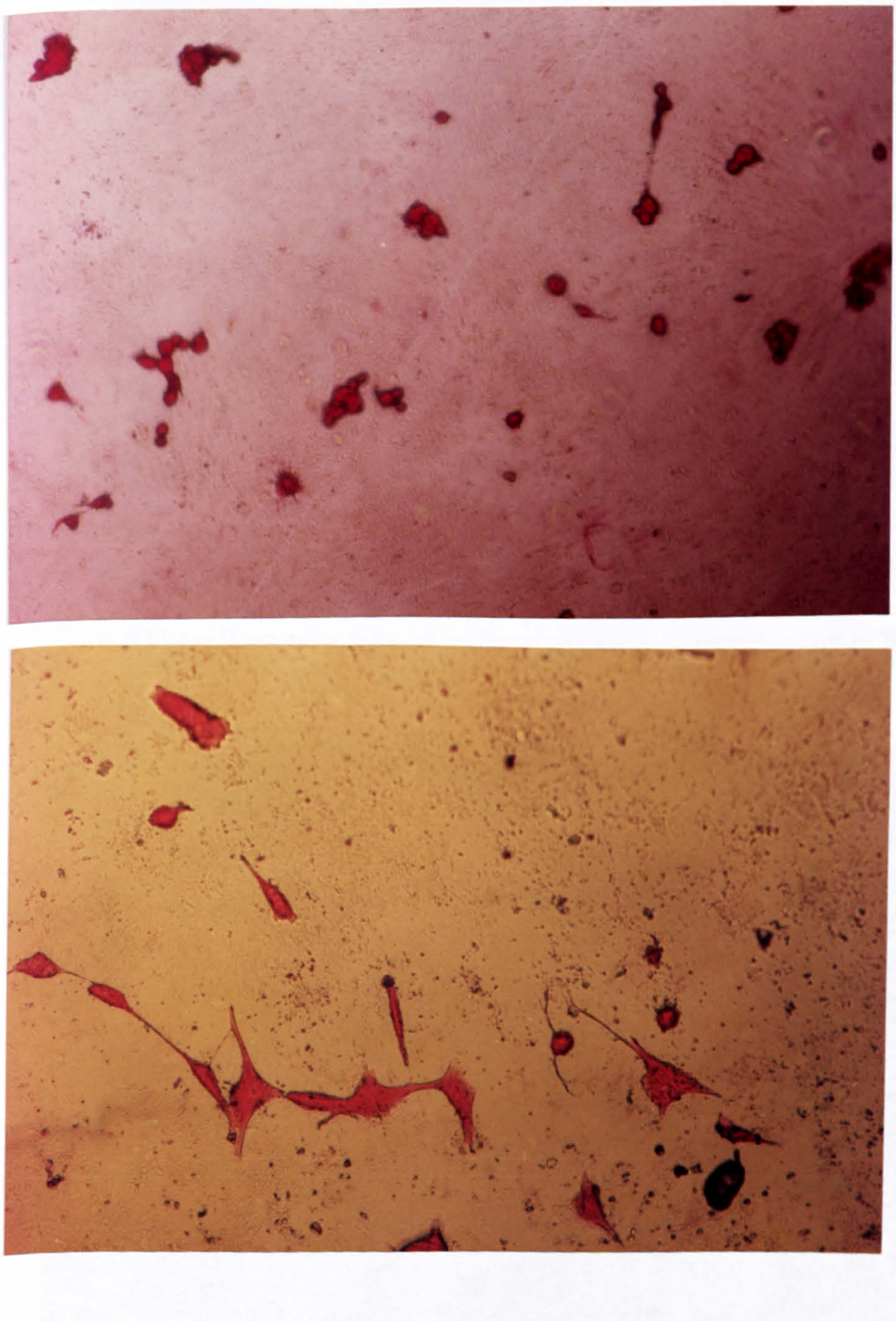


Figure 4.31 Optical micrographs of BHK cell attachment to laser treated PDMS at 9.58 μm wavelength, (a,b) cells stained with eosin (magnifications are 200 x).

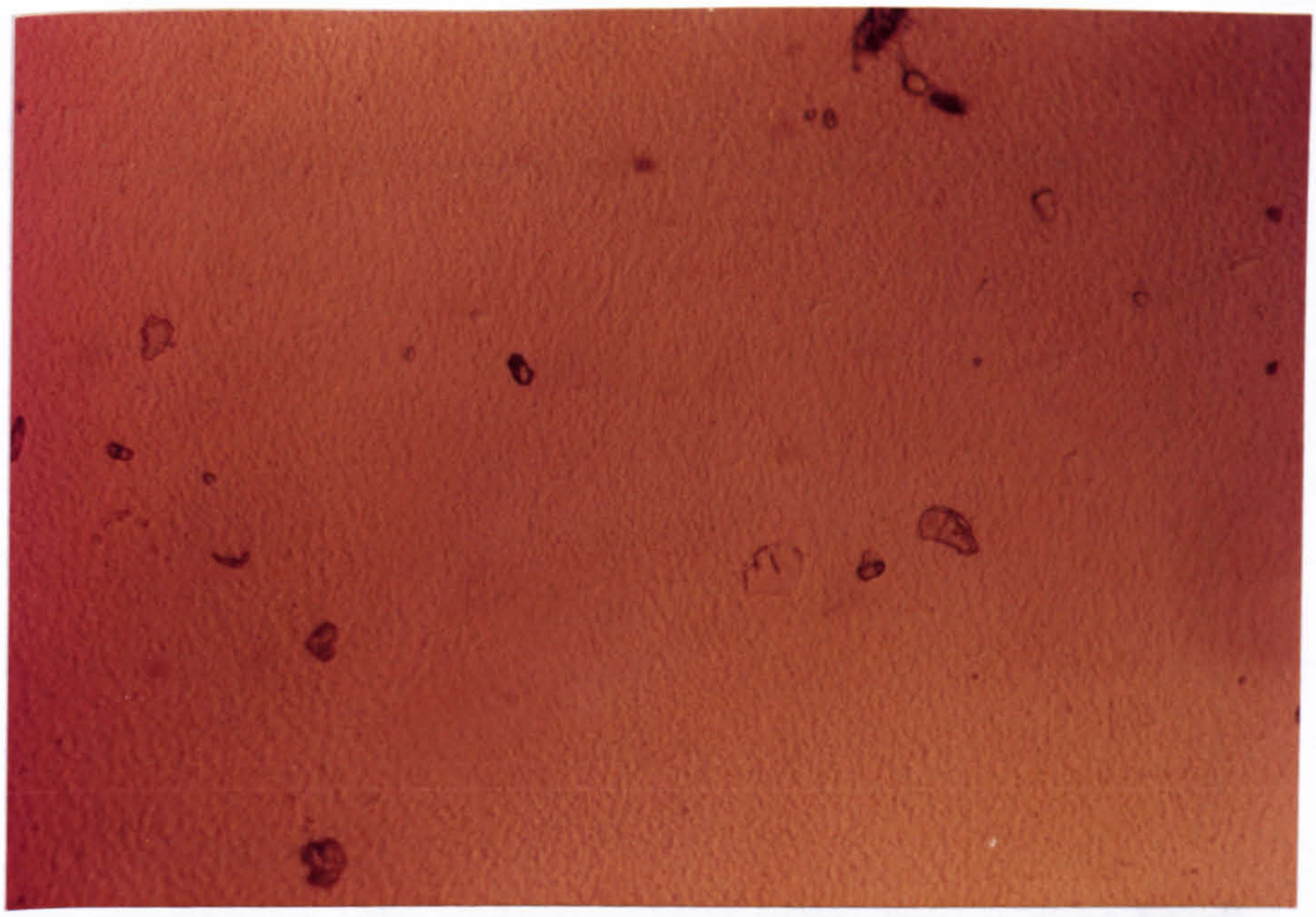


Figure 4.31 **Optical micrographs of BHK cell attachment to laser treated PDMS at 9.58 μm (1043 cm^{-1}) wavelength: (c) without staining (magnification is 200 x).**

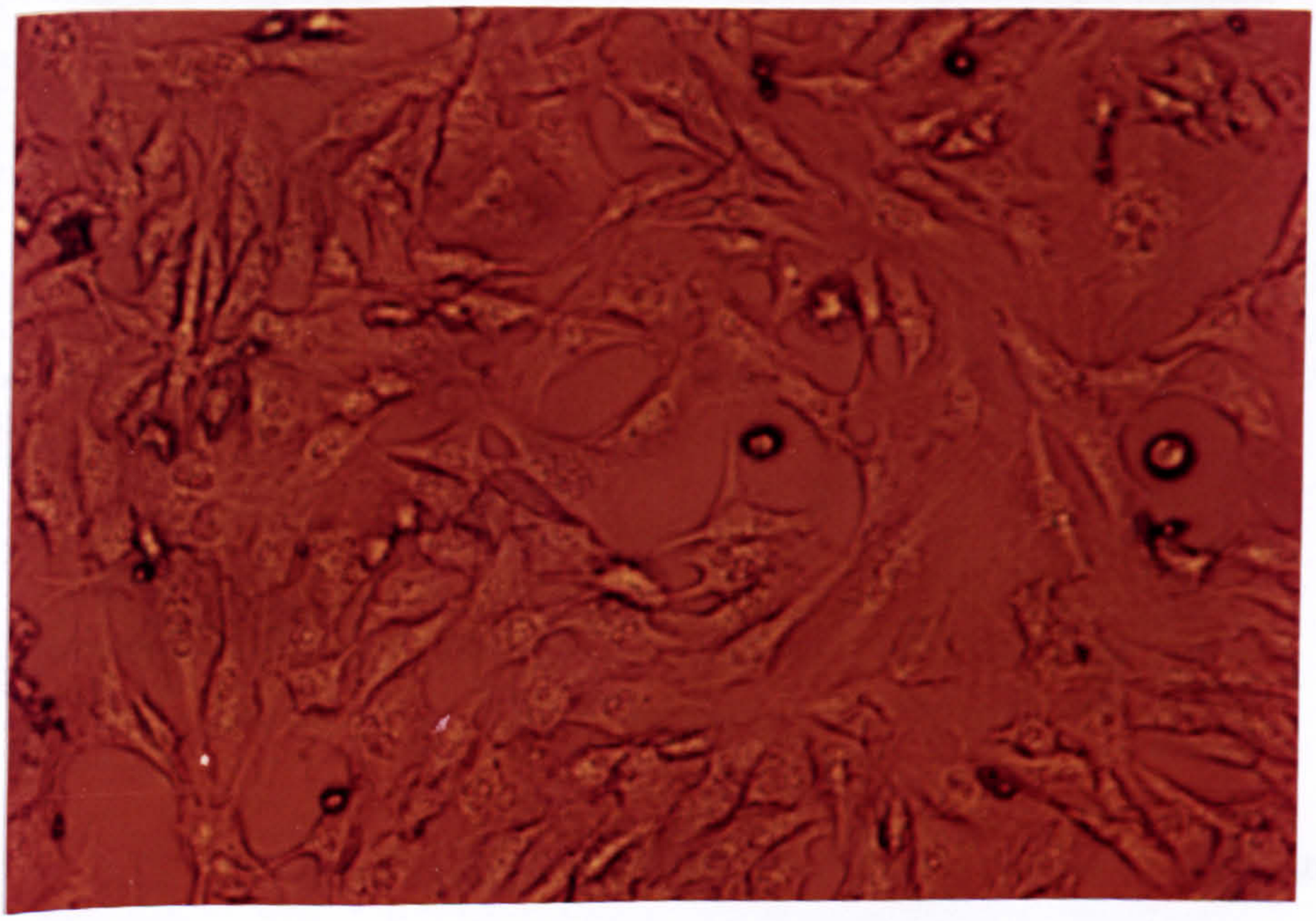
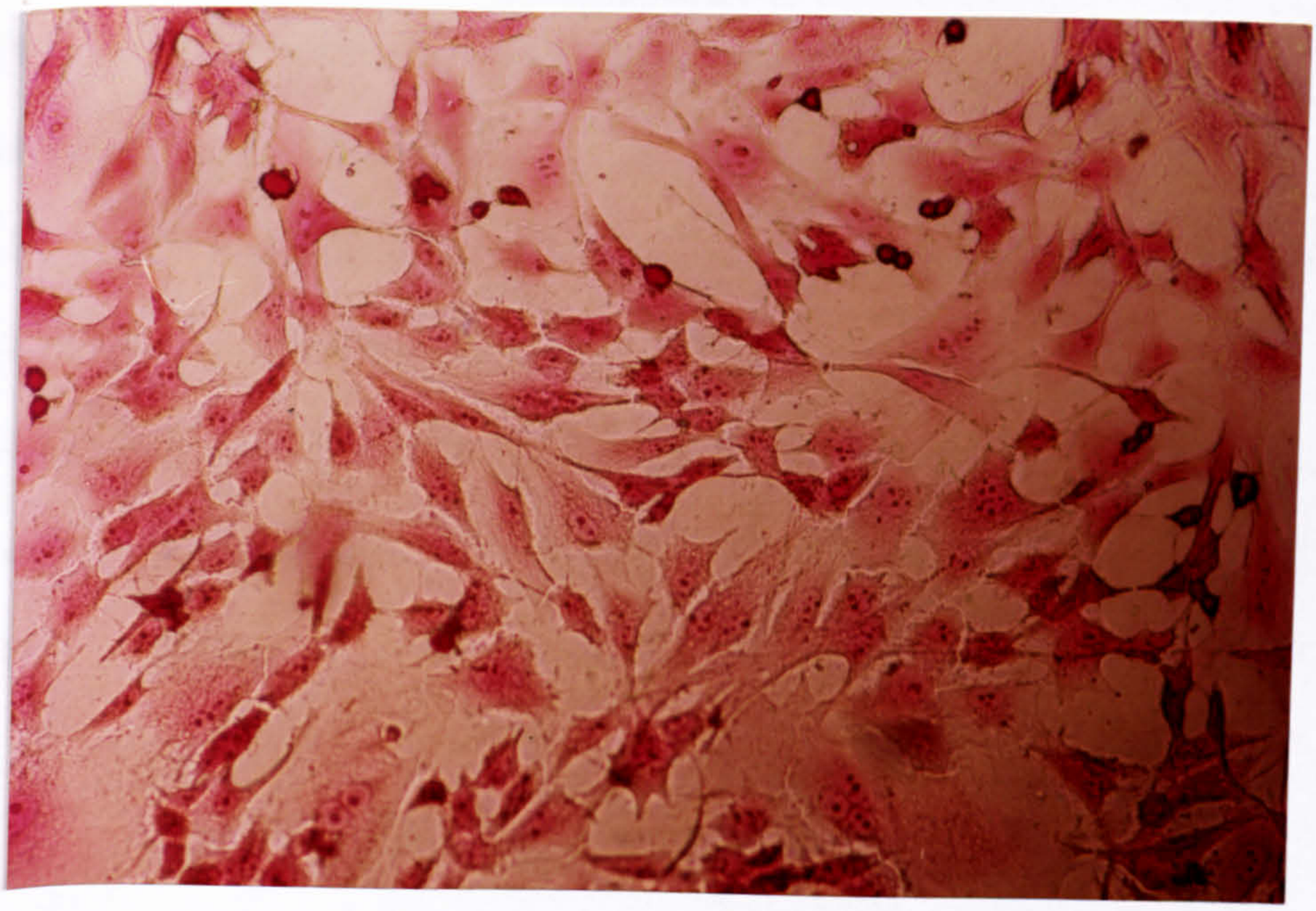
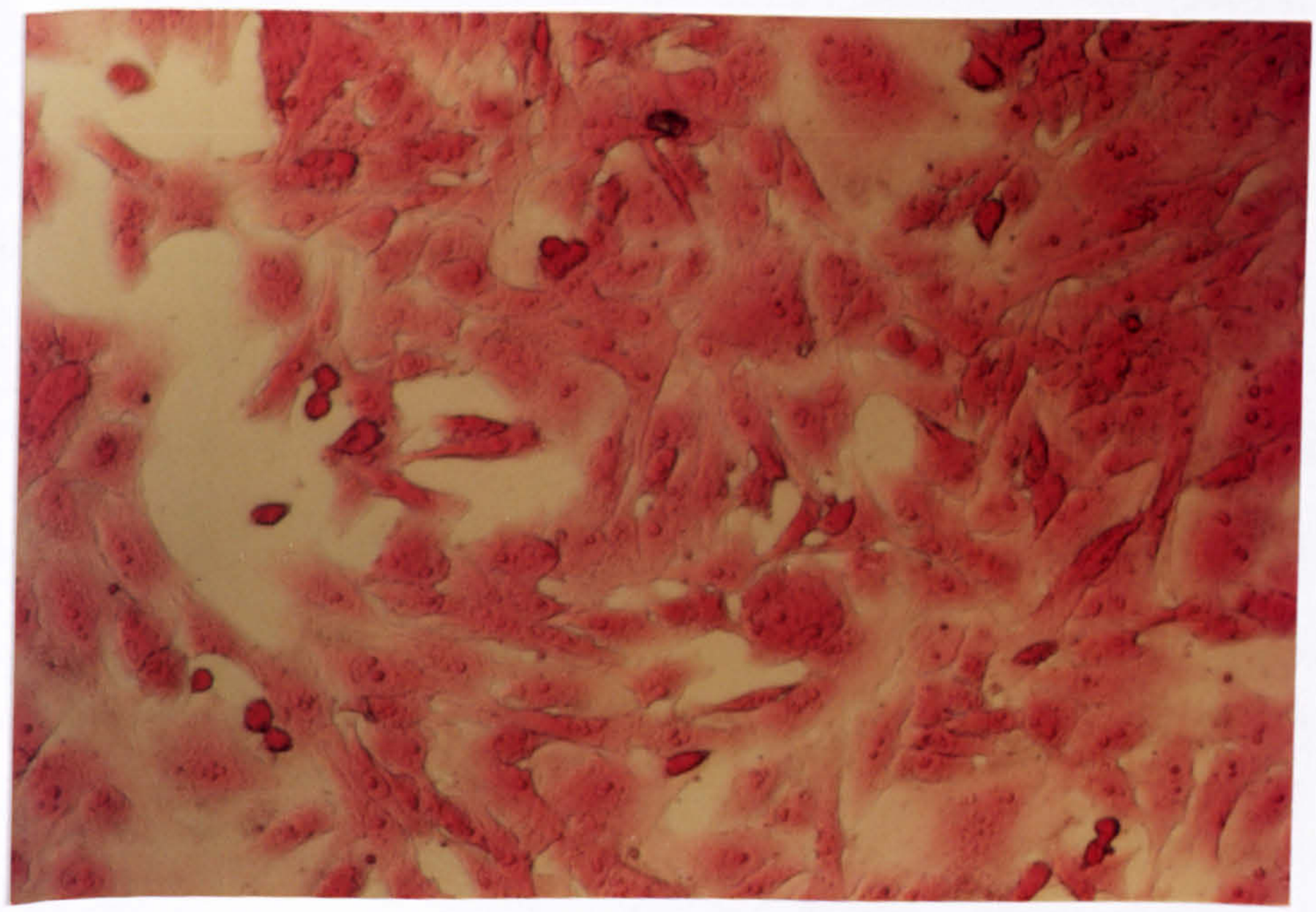


Figure 4.32 **Optical micrographs of BHK cells attachment to: (a) untreated PDMS (magnification is 200 x).**



(b)



(c)

Figure 4.32 Optical micrographs of BHK cells attachment to: (b) untreated PDMS which BHK stained with eosin; (c) glass which BHK stained with eosin (magnifications are 200 x).

Table 4.5 gives the comparative BHK attachment ratios for PDMS based surfaces. These were measured by fully detaching the cells from the surface by using trypsin, which causes the cells to be released into the culture medium.

As can be seen in these optical photographs, both the shapes and the numbers of the attached cells are different, depending on the substrate. The values for the attached cells are reported as a percentage of the total cells available for attachment. As shown in this table, fibroblastic cells (*i.e.* BHK cell) attachment is lower in the treated PDMS than in the case of the control and glass substrates.

Table 4.5 Dependence of BHK cells (9 BHK 21 P 188) attachment on the surfaces of glass, treated and untreated PDMS after detachment of adhered cells into the culture medium (number of cells in initial cell solution is 1.78×10^5/ml).

Sample	Adhered cell (cell/ml)	% of adhered cells
Glass	1.45×10^5	81
Untreated PDMS	4.25×10^4	24
1 pulse treated PDMS	2.5×10^3	1.4
5 pulses treated PDMS	2×10^3	1.1
15 pulses treated PDMS	1.15×10^4	6.5

The comparative results show more BHK cell adhesion and spreading on the glass and the untreated PDMS (smooth nonporous surfaces) than on all the treated PDMS surfaces (rough porous surfaces). The cells which adhered to the rough PDMS treated surfaces were round and the cell edges did not appear attached and filopodia growth was minimal. BHK cells on the smooth untreated PDMS and glass showed very flattened cells with small peripheral filopodia and ruffled edges. It seems that the CO_2-pulsed laser irradiation causes a significant decrease in the cell attachment, which is attributed to the high hydrophobic and porous morphology. This *in vitro* assay reconfirmed the results which we have previously discussed in section (4.3.1) regarding to the reduction of platelet adhesion onto the same surfaces. A pronounced change in the degree of cell adhesion was seen by large increase in pulse number. However the adhered cells on these surfaces showed abnormal behaviour *i.e.* the cell shapes become small and round but that, after detaching the cells into the culture medium, they return to the initial form, which indicates that the adhered cells have not died and only change their shape during the attached state.

The control PDMS surfaces showed much better growth and spreading properties than the treated PDMS surfaces. The surface topography of a material probably affects cells through `contact guidance'. Contact guidance refers to a directional cellular response to some property of the substratum the cell rests on[212].

Cell adhesion onto a material surface can be arbitrarily classified as a two-step mechanistic process: the first stage is controlled by complex combinations of physico-chemical interactions including hydrophobic, Coulombic, and Van der Waals forces between the cell membrane and the material surface. This process might be termed

" passive adhesion " according to this adsorption mechanism. The second stage might be considered as active adhesion, because of the participation of cellular metabolic processes. Attached cells are well-known for changing their shapes and expending metabolic energy in order to stabilize the interface between their membrane and the underlying materials, by both physico-chemical and biological mechanisms[213].

Figure 4.33 represents cell adhesion and detachment data on material surfaces. Cells are small particles but are distinctly different from small artificial particles in adhesion because cells have metabolism. After the cells contact surfaces (passive adhesion), the cells are always dynamically altering their cell membrane and its morphology to optimize interactions and to stabilize the cell-material surface interface (active adhesion), both physico-chemically and biologically. Therefore, cell adhesion should be divided into two stages: passive adhesion and active adhesion, as shown in Figure 4.33.

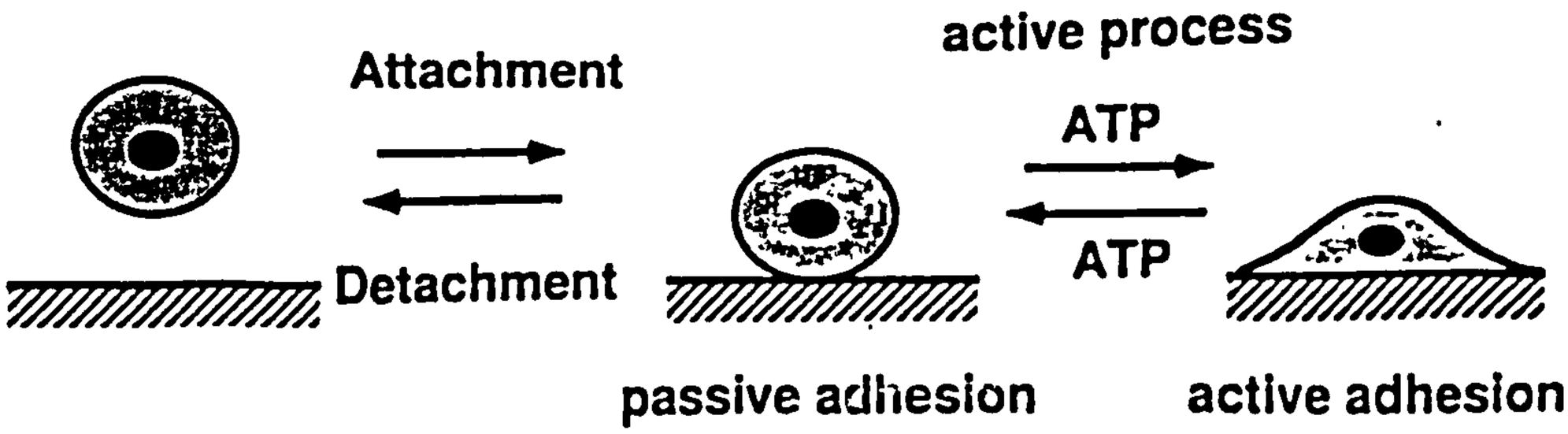


Figure 4.33 Mechanism of the cell attachment to and detachment from material surface.

These observations strongly suggest that the chemical and / or physical structure of the substrate controls the degree of cell adhesion. The results presented here demonstrate that PDMS surfaces can be successfully treated to create various surface structures which significantly influence the *in vitro* attachment of BHK fibroblast cells. Cell attachment for fibroblasts was statistically lowest for the rough PDMS treated surface. It is proposed that the interactions of the cell with the treated PDMS which are more hydrophobic is lower than that with the untreated PDMS. In conclusion, by this novel technique it is possible to reduce the protein absorption onto the irradiated PDMS surfaces (luminal surfaces or intima of the artificial vascular grafts) while the unmodified surfaces (outer surfaces) will remain tissue compatible, which is necessary for biocompatibility. This *in vitro* assay reconfirmed the results which we have previously discussed in section (4.3.1) regarding to the reduction of platelet adhesion onto the same surfaces.

4.3.3 Platelet adhesion study onto the AAm grafted PDMS

Platelet adhesion experiments were carried out *in vitro* using the PRP method. Scanning electron microscopy was used to study the morphology of the adherent platelets and to compare the platelet shape change responses on the different surfaces. SEM showing the morphology of platelets attached on the polymer surfaces are shown in Figure 4.34. Both low and high magnifications are shown. However, platelet spreading on the AAm grafted PDMS is less than that on the reference sample (untreated and ungrafted) [see figure 4.29]. Complete spreading and aggregation of the attached platelets are observed on the reference sample. However, no platelet spreading

and aggregation is observed on laser treated PDMS. It can be concluded from these studies that the surface of the laser treated PDMS do not induce platelet activation.

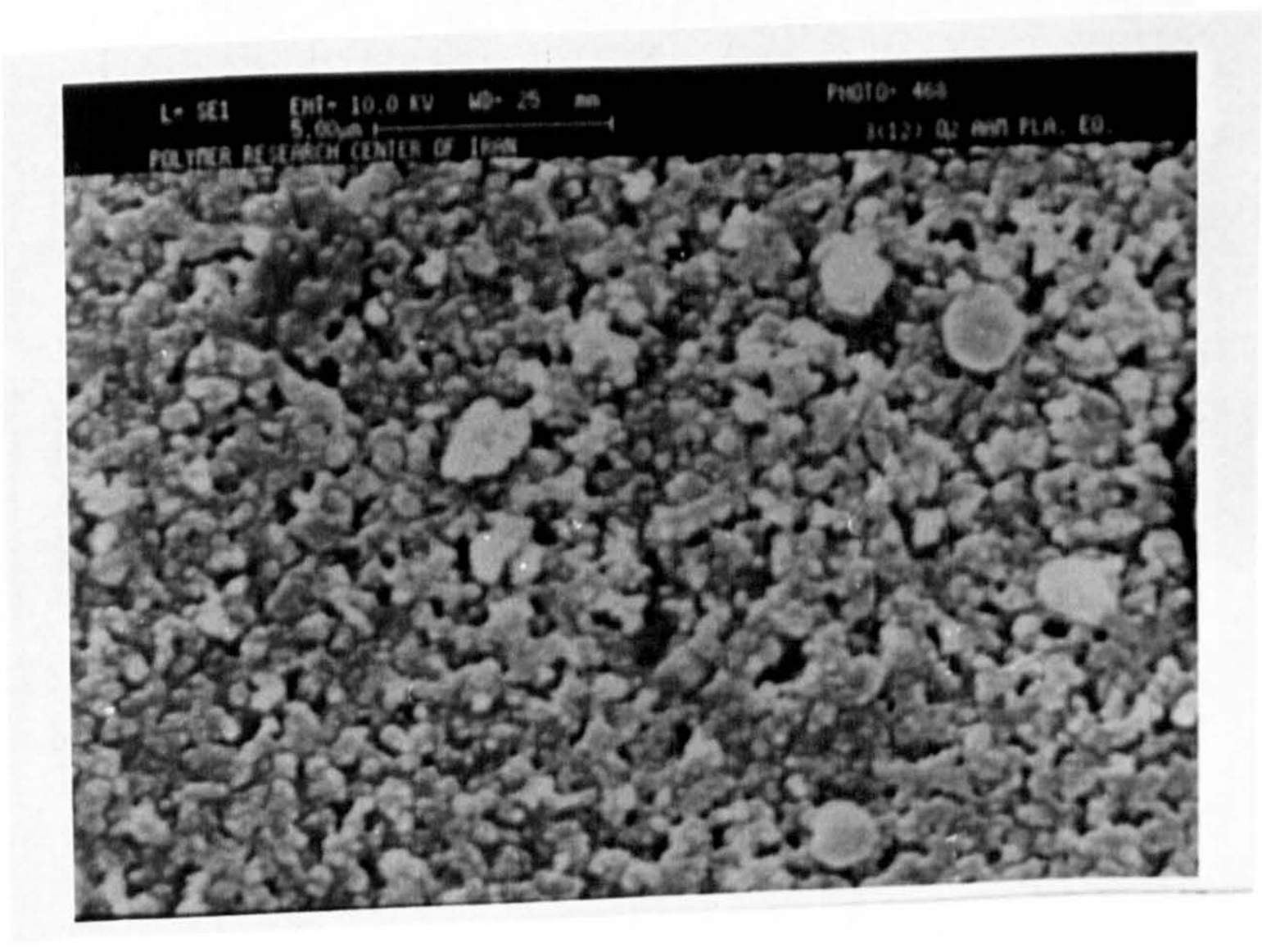


Figure 4.34 (a) SEM micrograph (magnification is 5000 x) of adhered platelets on the AAm grafted PDMS surface with CO_2-pulsed laser.

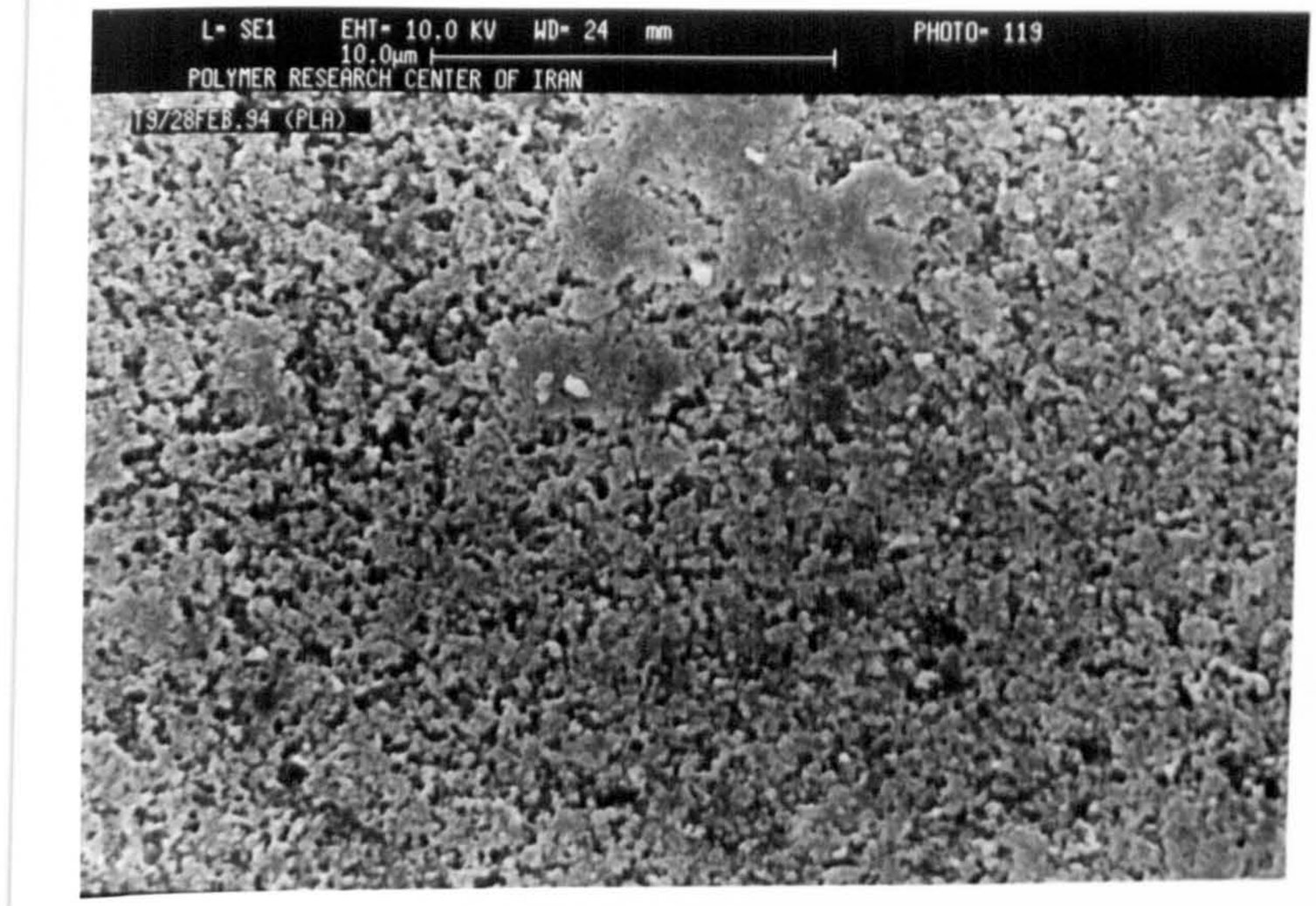


Figure 4.34 (b) SEM micrograph (magnification is 2000 x) of adhered platelets on the AAm grafted PDMS surface with CO_2-pulsed laser.

Traditionally the number of adherent platelets has been measured as a parameter for surface thrombogenicity in most *in vitro* studies[194,214]. The number of platelets adhered on the untreated (control sample), laser treated and AAm grafted PDMS films from PRP was obtained from the lactate dehydrogenase (LDH) activity method, as shown in Table 6.

Morphological changes of the platelets during spreading on surfaces have usually been

determined by counting the number of platelets in various spreading stages, such as discoid, dendritic, partially spread, spread and fully spread forms[210,214].

Table 4.6 Dependence of platelet attachment on the surfaces of glass, untreated and treated PDMS and AAm grafted onto the PDMS surface, obtained from the LDH method.

Sample	Adhered platelets / mm^2
Glass	19055
Untreated PDMS	17949
1 pulse treated PDMS	6557
5 pulses treated PDMS	2591
10 pulses treated PDMS	2195
AAm grafted PDMS	3900

There were more platelets adhering to the untreated PDMS substrate than the treated and AAm grafted PDMS, *i.e.* by this measure the untreated PDMS is more platelet-activating than the laser treated material. When comparing the effects of the different PDMS samples on platelet activation, the 10 pulses laser treated PDMS sample had the least amount of platelet adhesion (2195 platelets / mm^2 ; 100 % round). The control

samples had an extraordinarily large amount of spreading and fully spread (99 % of total) platelets as well as the highest total number of adherent platelets (17949 platelets / mm^2; 99 % fully spread). In the consideration of platelet activation, the total number of adhered platelets is of interest but, of more importance, is the number of platelets in their last two stages of shape change. For the laser treated PDMS sample, 10 pulses provided the least platelet-activating sample. All of the AAm grafted PDMS samples were more activating than the laser treated PDMS (3900 platelets / mm^2) toward platelet activation. The reduced platelet activating characteristics shown by the AAm grafted onto the PDMS compared to the untreated PDMS, could be due to the distinctive chemical (hydrophilic nature) and physical (morphology) properties associated with the PAAm formed on the surface. According to Ikada et.al[14], there are two possibilities for a polymer surface to have $W_{12,w}$ of zero, in other words, to be nonadhesive. One is to create a superhydrophilic, in other words, water-like surface (γ_{1w} =0) and the other is superhydrophobic one (γ_{1w} =73 $erg.cm^{-2}$). These two extreme types of surfaces are totally different each other but are both relatively blood-compatible [$W_{12,w}$ is the work of adhesion in water or in aqueous media between body 1 and body 2 and γ_{1w} is the free energy of the interface between body 1 and water]. There is another postulated model of the grafted outermost layer in contact with aqueous suspension of platelets. It is evident that platelet adhesion is greatly reduced by the surface graft polymerization of AAm. The grafted polymer chains may prevent the protein molecules and platelet cells from direct contact with the PDMS surface owing to their steric hindrance effect.

The ideal biomaterial would be the one which does not allow platelets to adhere at all.

As suggested, the transformation of contact-adherent platelets to the fully spread form on biomaterials is the first step toward thrombus formation[214].

4.3.4 Cell culture study onto the AAm grafted PDMS

The attachment of BHK (Baby Hamster Kidney) cells onto the AAm grafted surfaces has been observed by light microscopy. Photographs of BHK cells attached to the AAm grafted silicone rubber surfaces is compared in Figure 4.35. The attachment of the cells onto the laser-treated silicone surfaces have been found to be negligible, whereas, as described in the previous section, there is an enhanced attachment of BHK cells to the untreated and ungrafted (control) silicone surface. Cell attachment to the laser-induced AAm grafted silicone rubber surface is found to be higher when compared to the CO_2-laser treated PDMS surface, as shown in Figure 4.35, but lower when compared to the control PDMS surface.

This *in vitro* assay is similar to the results of platelet adhesion onto the same surfaces, showing the reduction of the protein adsorption onto the laser treated surfaces due to the decreasing of surface tension of the modified samples. Hence through this novel technique there is possible to modify the inner surface (intima) of the vascular graft to reduce the platelet adhesion while the outer surface is unmodified for cell proliferation. This is necessary for implant fixation into the tissues body.

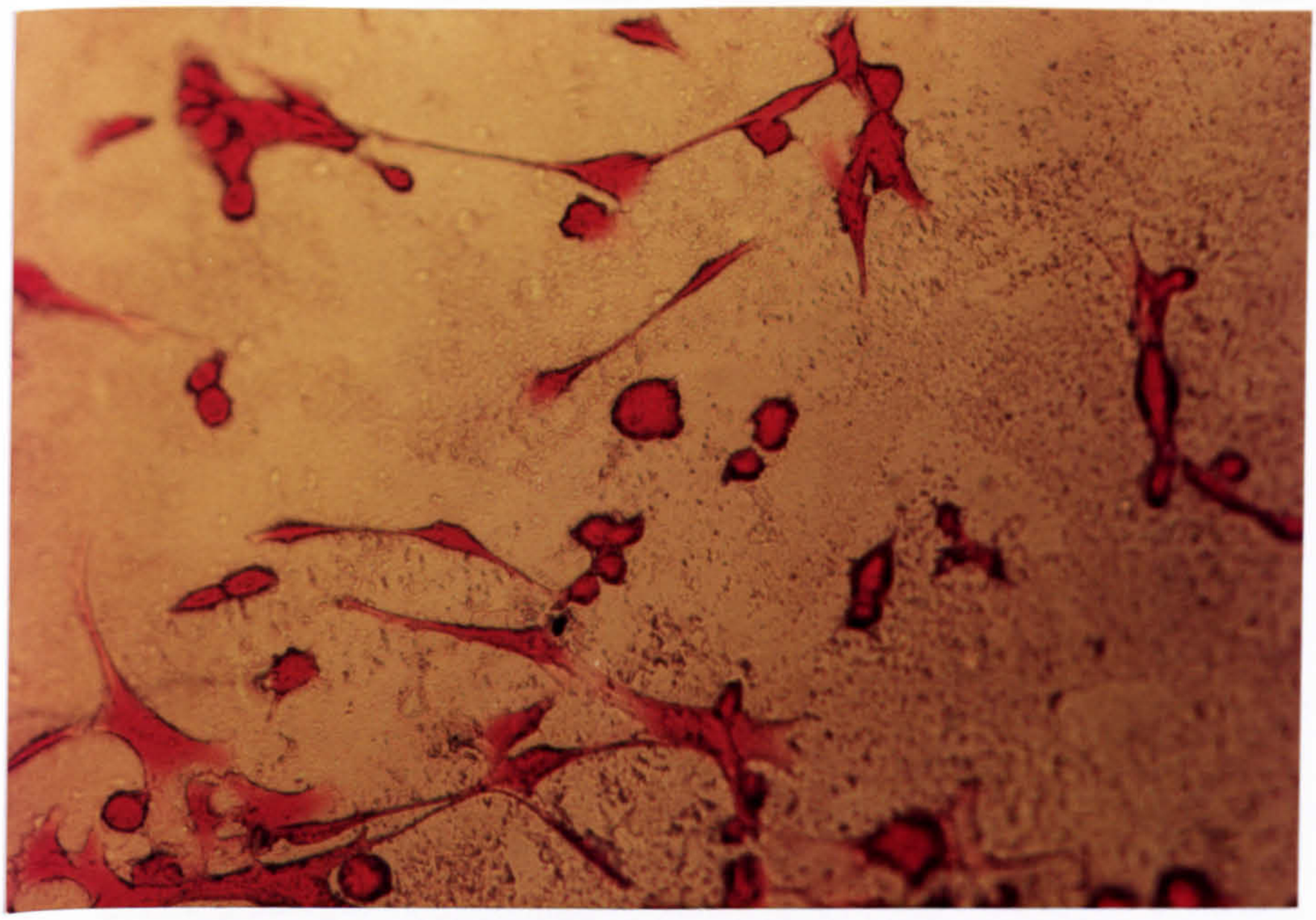


Figure 4.35 **Optical micrographs of BHK cells attached to AAm grafted PDMS, stained with eosin (magnification is 200 x).**

4.3.5 Platelet adhesion onto the HEMA grafted PDMS

Scanning electron micrographs (SEM) and photolight microscopy showing the morphology of platelets attached on the polymer surfaces are shown in Figure 4.36(a,b).

The number of platelets adhered onto the untreated and treated PDMS by laser and grafted PDMS with HEMA obtained from the LDH method are shown in Figure 4.37.

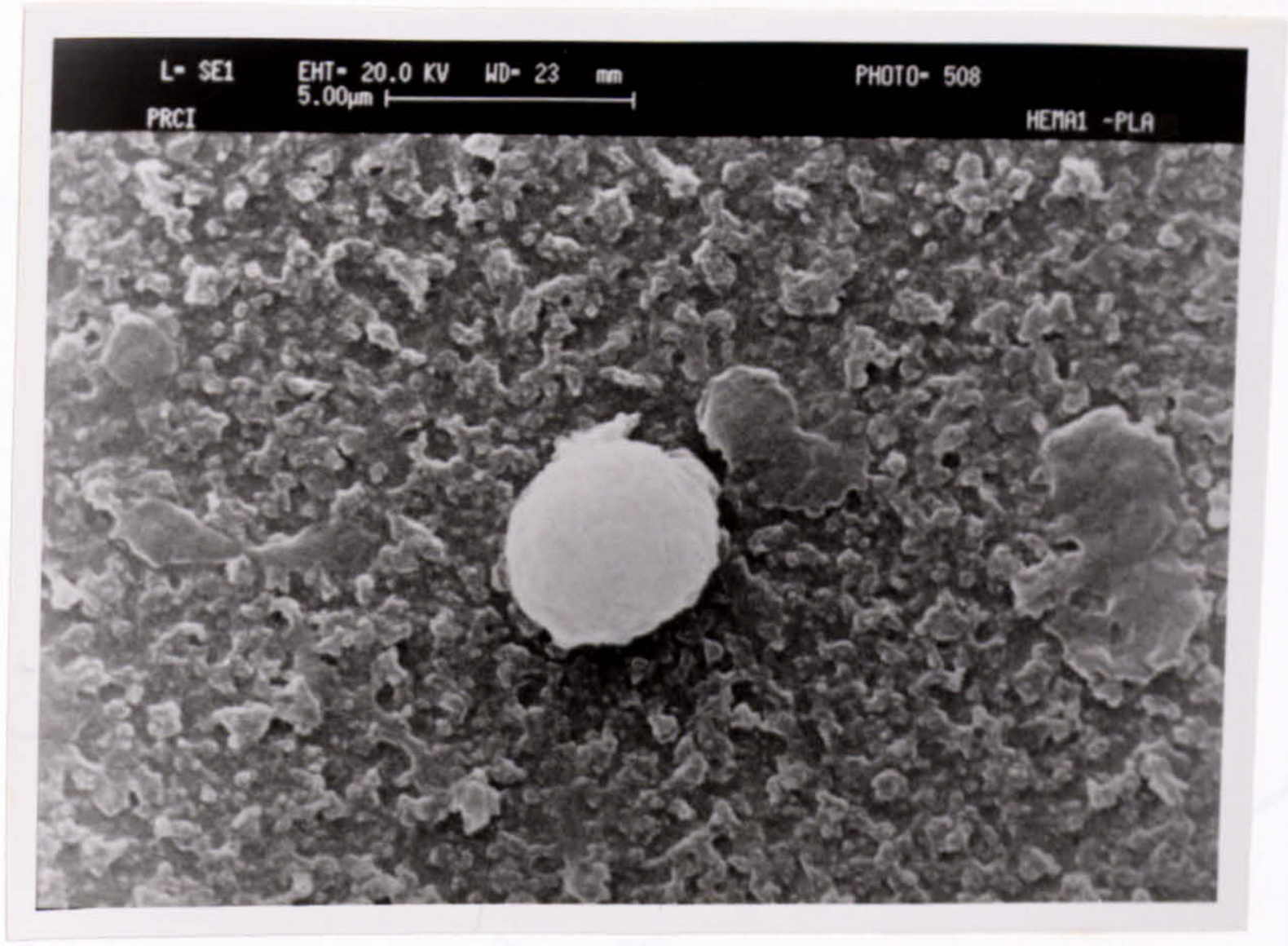


Figure 4.36 (a) SEM micrograph (magnification is 5000 x) of adhered platelets onto the HEMA grafted PDMS.

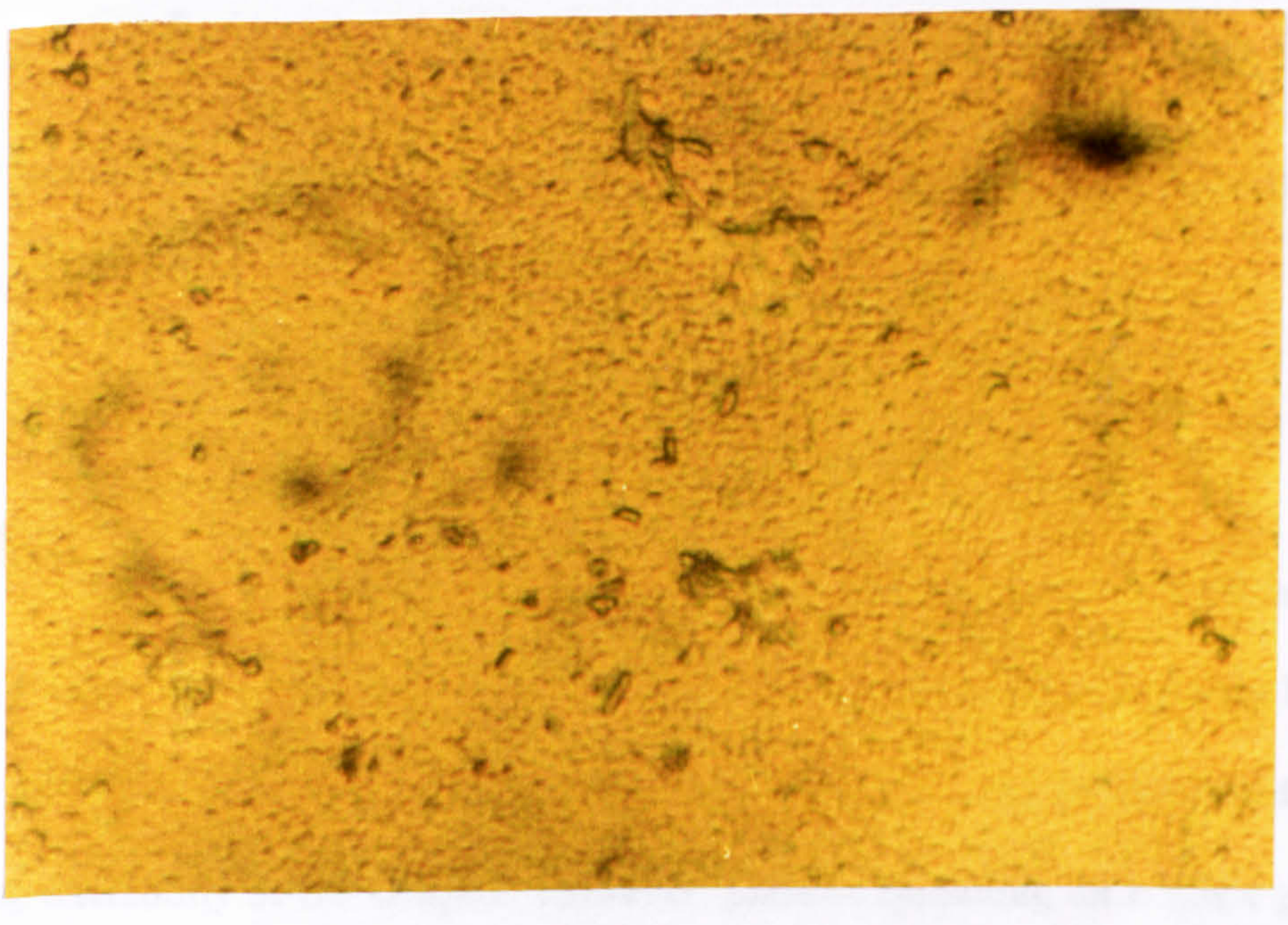


Figure 4.36 (b) Optical photomicrograph of platelet attachment to HEMA grafted PDMS (magnification is 1000 x).

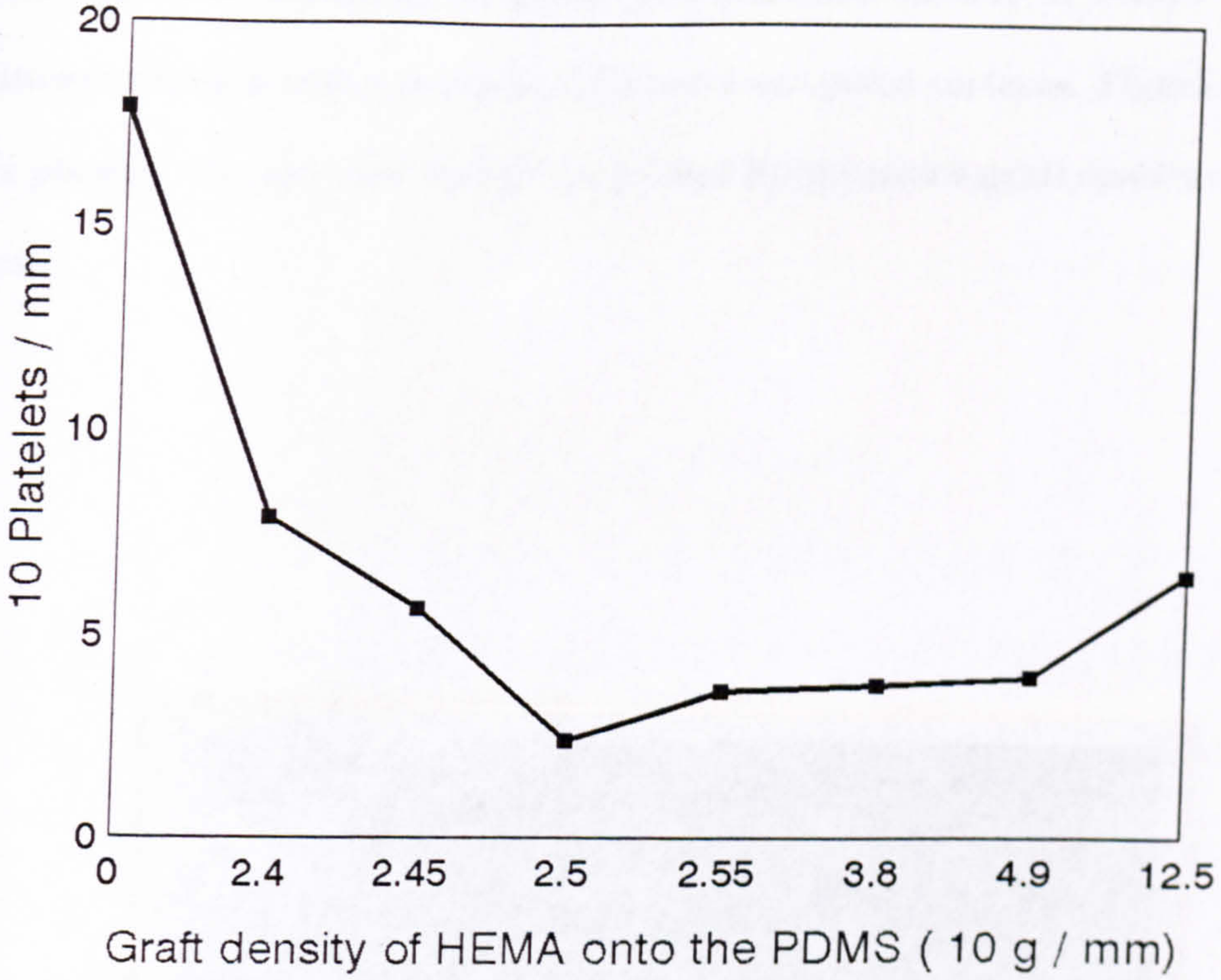


Figure 4.37 **Dependence of platelets adhesion onto the various concentration of HEMA grafted PDMS.**

As can be seen platelet adhesion is maximum on the untreated (control PDMS sample) while it is minimal on the laser-treated PDMS, whilst platelet attachment on the HEMA grafted PDMS seems to be intermediate between these. This can be attributed to the different wettability of the samples. However, platelet spreading on HEMA grafted PDMS is less than that on the reference sample (untreated and ungrafted). Complete spreading and aggregation of attached platelets are observed on reference sample. The

HEMA grafted surface of PDMS showed platelets which had numerous microvilli, fads, some blebs and extending filopodia. The untreated surface of PDMS contained flattened platelets with some radical filopodia and pilled surfaces. Figure 4.38 shows the platelet adhesion onto the HEMA grafted PDMS with a graft density of 6.5×10^{-3} g/cm^2.

Figure 4.38 SEM micrograph of platelet adhesion onto the HEMA grafted PDMS with graft density of of 6.5×10^{-3} g/cm^2.

By increasing the graft density, the topography of the surface became smooth and we can not see any porosity on it. As can be seen, platelet adhesion on it is higher than the lower graft density of HEMA onto the surface of PDMS (Figure 4.36). We conclude from this result that the morphology, especially rough surfaces, have a major effect on blood compatibility, which was also observed from the *in vitro* experiments.

4.3.6 Cell culture onto the HEMA grafted PDMS

The attachment of Baby Hamster Kidney (BHK) cells onto the HEMA grafted PDMS surfaces have been observed by light microscopy. Photomicrographs of BHK cells attached to the PHEMA- grafted silicone rubber surfaces stained with eosin are shown in Figure 4.39.

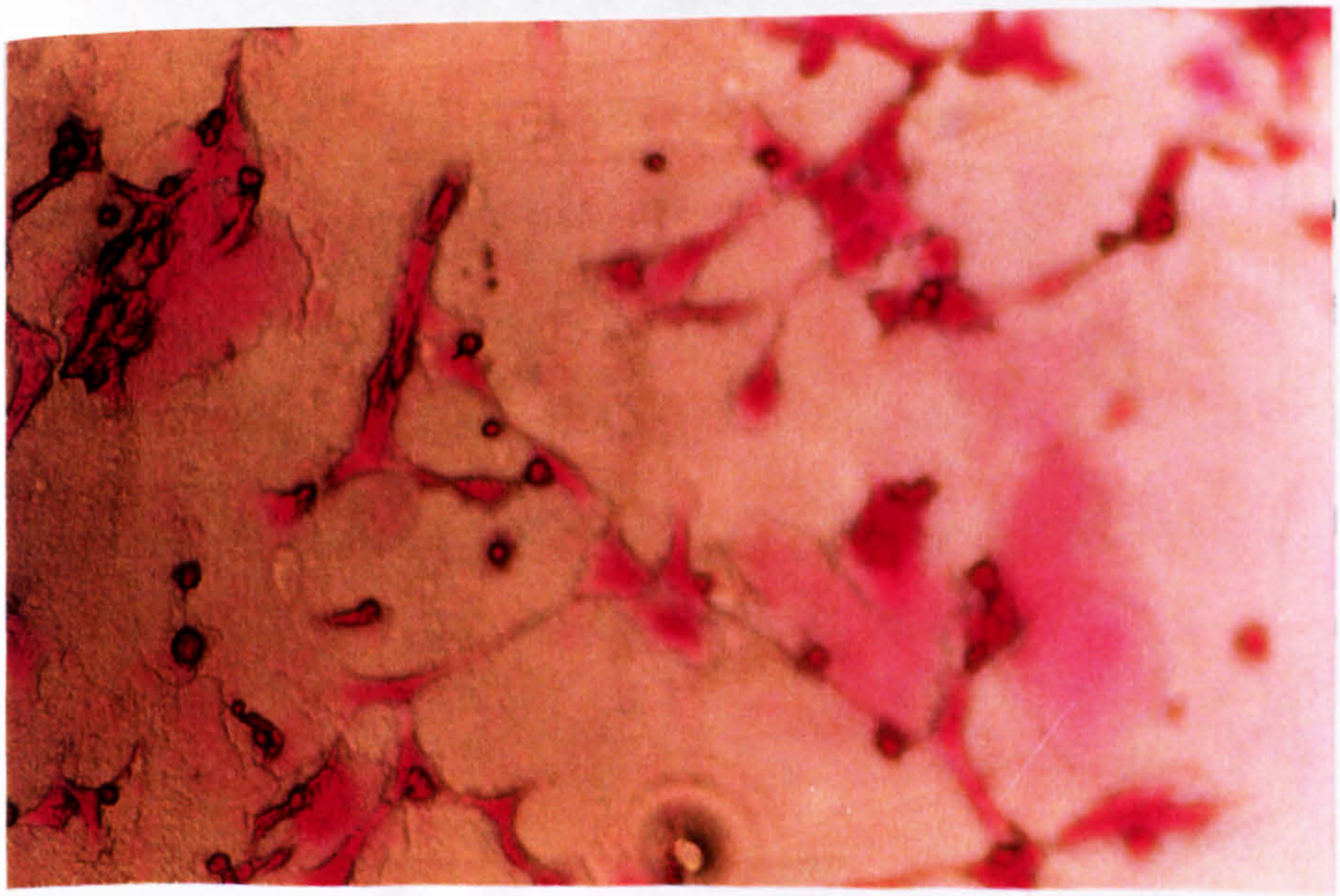


Figure 4.39 Optical micrograph of BHK cells attachment onto the HEMA grafted PDMS, stained with eosin (magnification is 200 x).

The number of cells attached to the laser-induced HEMA grafted silicone rubber surface is found to be higher when compared to the CO_2-laser treated PDMS surface, as shown in Figure 4.39, but lower when compared to the control PDMS surface. This *in vitro* assay is consistent with the results of platelet adhesion onto the same surfaces.

4.3.7 Platelet adhesion study onto the HEMAPC grafted PDMS

Scanning electron micrograph and photolight micrograph showing the morphology of platelets attached onto the HEMAPC grafted PDMS surfaces are given in Figure 4.40(a,b).

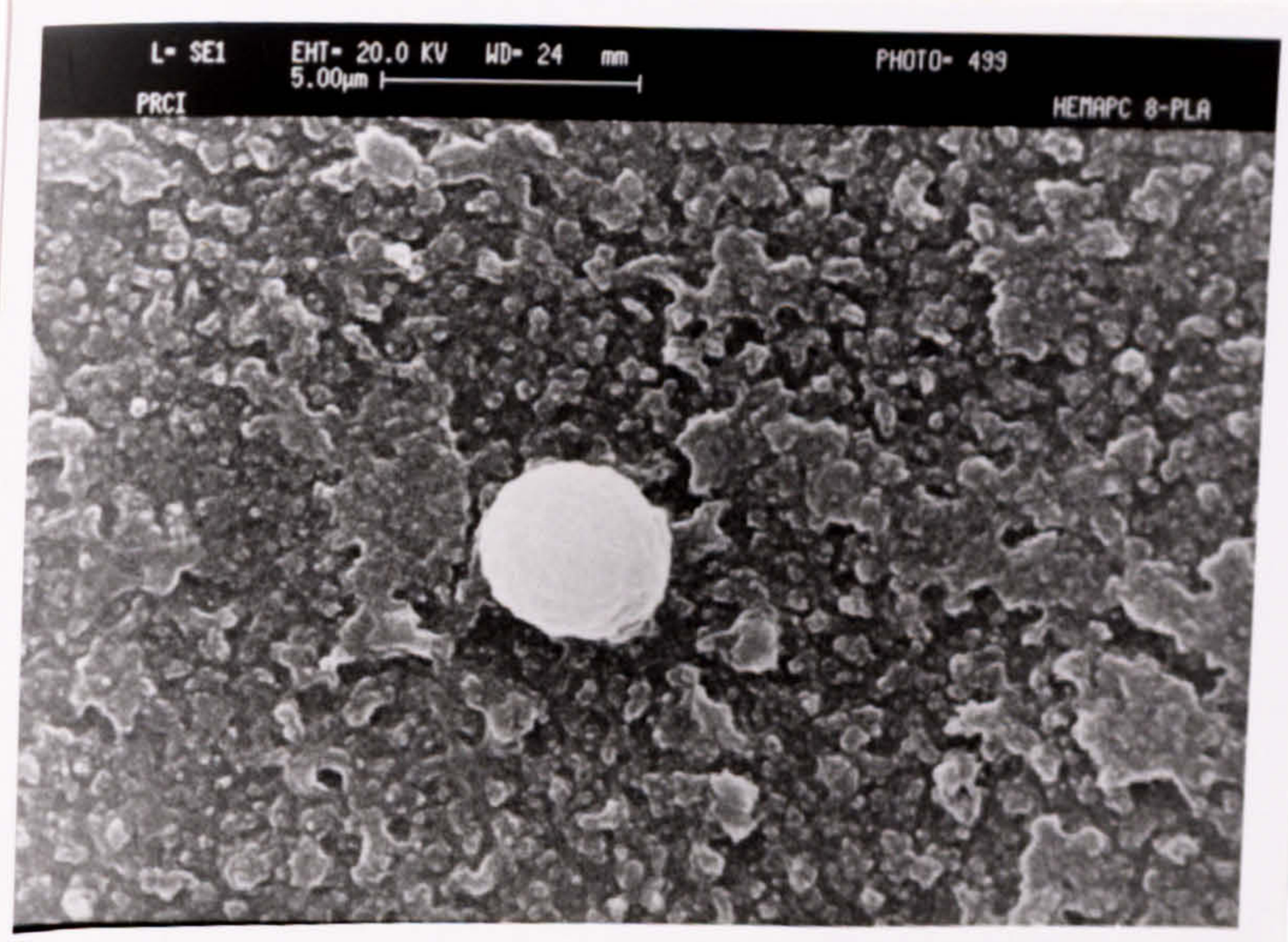


Figure 4.40 (a) SEM micrograph (magnification is 5000 x) of adhered platelets onto the HEMAPC grafted PDMS.

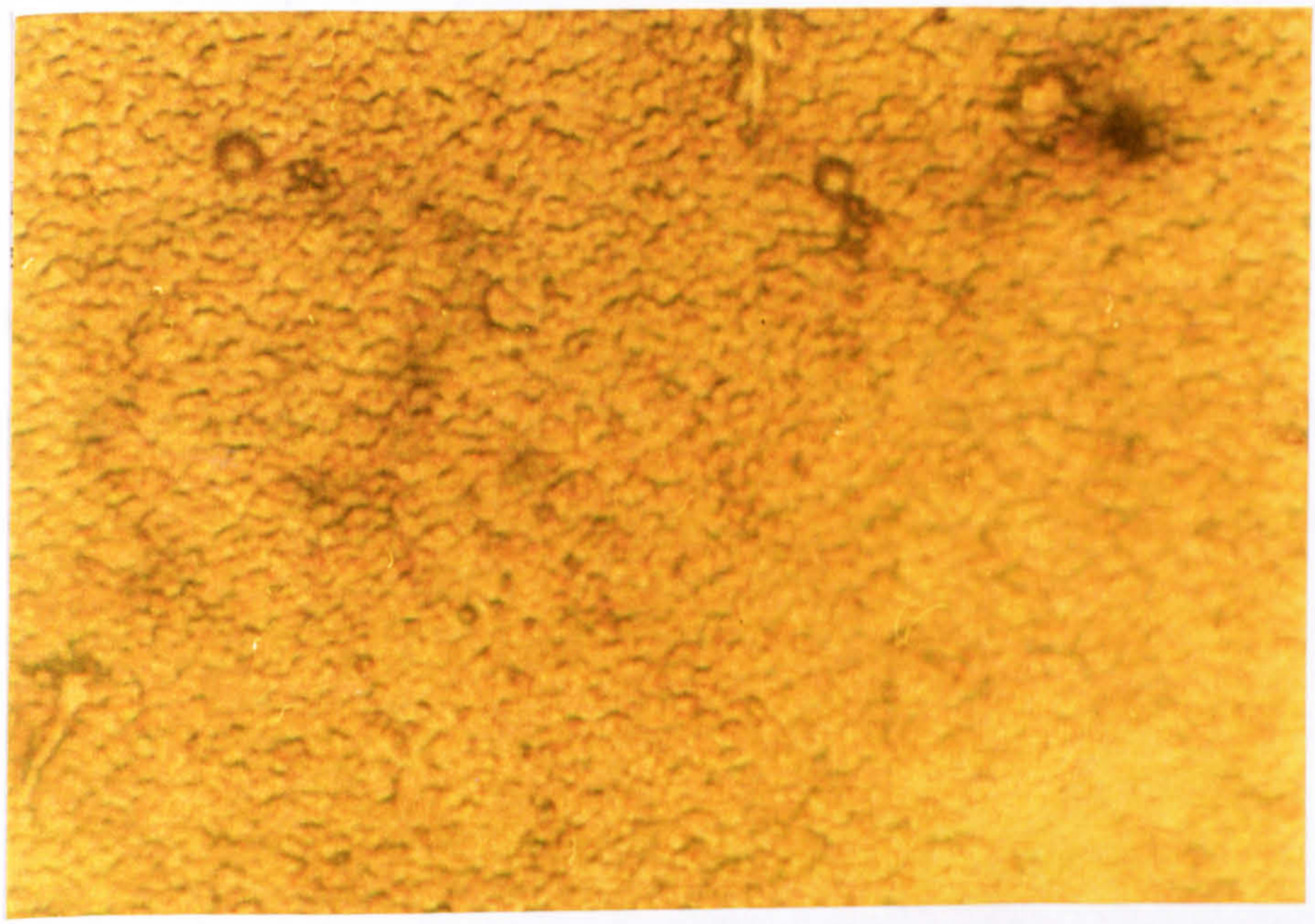


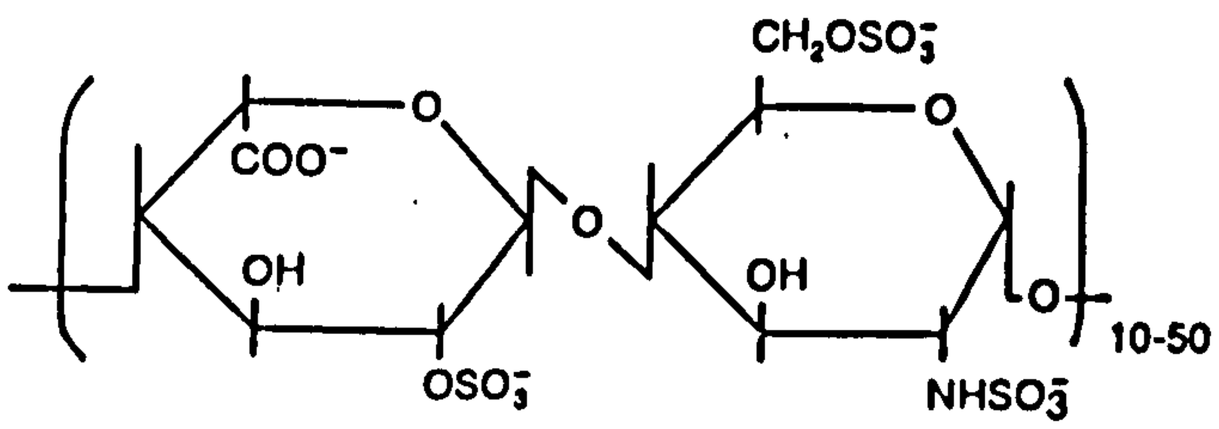
Figure 4.40 **(b) Optical photomicrograph of platelet attachment to HEMAPC grafted PDMS (magnification is 1000 x).**

The surface was incubated with PRP under the standard experimental conditions for comparison. Both low and high magnifications are shown. Comparison of this surface with the control, HEMA grafted PDMS and AAm grafted PDMS materials indicate that the platelet adhesion on HEMAPC grafted PDMS is much less than on the other surfaces. Most of the platelets on this surface retain their shapes and are small and round but those on AAm and HEMA grafted PDMS extend small pseudopods. Platelet spreading on HEMAPC grafted PDMS is less than that on the reference (control

sample) and AAm, HEMA grafted PDMS.

It can be concluded from these studies that the surface of HEMAPC grafted PDMS does not induce platelet activation. For better comparison, all of the acrylate grafted PDMS and control PDMS and laser treated PDMS without grafting were estimated by measuring lactate dehydrogenase (LDH) activity after detaching of attached platelets. However, because attached platelet number counting is one of the most popular method used for evaluating the hemocompatible properties of man made materials[1,4,14,134-136,162] , this method was also used. The results are shown in Table 7. As can be seen, ungrafted CO_2-pulsed laser treated PDMS surfaces and HEMAPC grafted PDMS surfaces have better results for blood compatibility, compared to the other treatments. These two surfaces do not induce platelet activation and the number of platelets attached are fewer than the control and HEMA and AAm grafted PDMS samples.

It is necessary to discuss these observations in this section. As mentioned in section (4.1.4) there are some factors that affect blood compatibility. The most important factors are surface roughness, the surface wettability (hydrophilic and hydrophobic) as discussed in previous sections. One of the other factors that could affect its properties is the charge on the surface; negative charge surfaces are believed to improve blood compatibility. The surface of intima of blood vessels is negatively charged (1-5 mv) with respect to the adventitia[13]. The chemical nature of the surface of a polymer, *i.e.* the exposed functional groups, determines the type and magnitude of the surface charge. The surface of the intima is negatively charged largely due to the presence of polysaccharides, especially chondroitin sulfate and heparin sulfate. Heparin is a polysaccharide with negative charges due to the sulfate groups as shown:



L-iduronic acid D-glucosamine

Typical repeat structure of heparin.

Table 4.7 Comparison of various PDMS surfaces on blood compatibility.

Samples	Platelets / mm^2
Glass	19055
Unmodified PDMS	17949
Laser treated PDMS (1 pulse)	6557
laser treated PDMS (5 pulses)	2591
laser treated PDMS (10 pulses)	2195
Acryl amide grafted PDMS (graft density is 2.45 x 10^{-3} g/cm^2)	3900
HEMA grafted PDMS (graft density is 2.5 x 10^{-3} g/cm^2)	2401
HEMAPC grated PDMS (graft density is 2.45 x 10^{-3} g/cm^2)	3389
HEMAPC grated PDMS (graft density is 2.6 x 10^{-3} g/cm^2)	3000
HEMAPC grated PDMS (graft density is 2.65 x 10^{-3} g/cm^2)	2362
HEMAPC grated PDMS (graft density is 2.70 x 10^{-3} g/cm^2)	1992
HEMAPC grated PDMS (graft density is 6.9 x 10^{-3} g/cm^2)	3972
HEMAPC grated PDMS (graft density is 10.25 x 10^{-3} g/cm^2)	5593

As can be seen from the heparin formula, oxygen enriched groups and negative charges all have an effect on the blood compatibility of heparin. These groups seem to create a strong bond hydration shield around the molecules, which serve to prevent any molecular recognition process.

Considering the laser-treated PDMS surfaces, as discussed in section (4.3.1), both the roughness (porosity) and the changed chemical nature of the surface, becoming oxygen enriched, serve to alter the properties to non-platelet activating as compared to the nature of the platelet-activating untreated PDMS. The net effect of the oxygen-enriched nature of the treated PDMS may be to increase local hydration.

Grafting AAm, HEMA and HEMAPC onto the PDMS surfaces leads to materials oa a hydrophilic nature with polar bond-groups. Of the these grafted materials, neither AAm nor HEMA bear negatively charged groups. However, in each case the bond groups are highly hydrated. The HEMAPC-grafted material contains the phosphatidyl choline zwitterionic head group, which is known to be highly hydrated and is used in nature in forming phospholipid membranes, to avoid recognition from (bonding to) blood proteins and cells.

CHAPTER 5

CONCLUSION

1- Our results from CO_2 - pulsed laser treatment of the surface of PDMS indicate the potential of using CO_2- pulsed laser treatment to induce oxidation and also structuring of the surface of PDMS, without the need for a photosensitizer, therby changing the PDMS surface's chemical and physical properties. PDMS is surface modified by laser treatment providing it is irradiated by pulses having a wavelength where the PDMS has a strong IR absorption. We believe that the laser induced modification of the surface of PDMS at 9.58 μm (1043 cm^{-1}) wavelength occurs by the vibrational excitation of - Si - O - bonds through infrared multiphoton dissociation mechanisms (IRMPD).

2- The modified PDMS contains carbonate and the other resulting from oxidation groups such as hydroxyl and carboxyls which enrich the oxygen content of the surface. Treated samples showed significant variation in hydrophobicity.

3- Untreated PDMS has a smooth surface, which, after treating by laser, changes to a rough surface with micropores in a similar size range and are uniform on all of the exposed. The width of pores on the surface of laser treated PDMS in an oxygen atmosphere at 10 pulses are about 1 - 10 μm and the depth is about 1 - 5 μm. When the pulse number is increased, to 15 pulses, the homogeneity of porosity drops and at 15

to 20 pulses, the surface changes to a new morphology which has very little porosity.

4- By increasing the pulse number, the O/Si ratio on the treated surface is increased up to 5 pulses after which this ratio tends to a plateau state. The increased O/Si intensity ratio is evidence for oxidized groups formed by laser treatment.

5- The virgin PDMS surface film has a water drop contact angle of 105 degree. After treatment of PDMS by the CO_2 - pulsed laser, by only one pulse, the water drop contact angle changed to give a value of 170 degree. This means that the surface property of the treated samples has been changed and a highly hydrophobic surface is obtained compared with the unmodified PDMS.

6- Results show an insignificant change in the bulk mechanical properties of the laser treated PDMS. It can be concluded that the bulk structure and therefore the mechanical properties of the laser-treated samples have remained intact.

7- The friction coefficient of the untreated silicon is 0.4 but drastically decreases when the surface is laser irradiated, even if only a single pulse is delivered to the silicone surface. The friction coefficient is 0.011 when the silicone surface is treated by 10 pulses in an oxygen atmosphere. The trend of decrease of friction coefficient of the laser treated PDMS in air and nitrogen atmosphere versus pulse number is the same as in oxygen, but in air is lower than oxygen and in nitrogen atmosphere is lower than air.

8- Acryl amide (AAm), 2-hydroxyethylmethacrylate (HEMA) and hydroxyethylmethacrylate phosphatidyl choline (HEMAPC) were grafted onto the PDMS surface.

9- Peroxides are formed to a maximum concentration of $7x10^{-7}$ mol cm^{-2} by only one pulse of the CO_2- laser, and then decrease by increasing the pulse number.

10- The characteristic absorption bonds of AAm ($-CH_2-C-C-NH_2$) appearing at 1609 - 1673 cm^{-1} and 3220-3400 correspond to the grafted carbonyl and amine groups of AAm respectively. These groups did not appear in the spectrum of the unmodified sample.

11- The characteristic absorption bonds of PHEMA appearing at 1713-1718 cm^{-1} and 3335 cm^{-1} correspond to the grafted carbonyl and hydroxyl groups of PHEMA . Also absorbance of characteristic peaks of PDMS such as (-Si-O-) and (Si-C) bond in the grafted PDMS decrease compared to the unmodified PDMS.

12- a maximum graft density was obtained using one pulse laser beam and 30 wt % acrylate monomer (AAm, HEMA) solution.

13- Characteristic absorbance bonds of HEMAPC appearing at 3337 cm^{-1}, 1717 cm^{-1} and 1481 cm^{-1} contribute to the $-N^+Me_3$ and carboxyl groups and also absorbance of PDMS bonds were decreased.

14- Comparison between control, AAm, HEMA and HEMAPC modified silicon rubber surfaces show that the rough surface of a AAm, HEMA and HEMAPC modified silicone rubber are homogeneous.

15- The measurement of the water drop contact angle of the AAm, HEMA, HEMAPC grafted surfaces show that the contact angle decreased and a hydrophilic surface was obtained. The water drop contact angle of HEMAPC grafted onto the PDMS surface by a single laser pulse and 5 wt % monomer concentration is about 10°.

16- Data from *in vitro* blood compatibility experiments indicated a significant reduction of platelet adhesion and aggregation for the laser treated surfaces and that those platelets, which were adherent, remained unspread. The extent of platelet adhesion was correlated with the number of laser pulses.

17- In vitro results (PRP and LDH activity methods) show that the platelet adhesion is at a maximum on the untreated PDMS while it is at a minimum on 10 pulses laser-treated samples.

18- CO_2-pulsed laser irradiation causes a significant decrease in the attachment of BHK cells which is attributed to the high hydrophobic and porous morphology. This *in vitro* assay reconfirmed the results of platelet adhesion.

19- In conclusion, by this novel technique it is possible to reduce the protein absorption onto the irradiated PDMS surfaces (luminal surfaces or intima of the artificial vascular grafts) while the unmodified surfaces (outer surfaces) will remain tissue compatible, which is necessary for biocompatibility.

20- Data from in vitro results show that platelet adhesion is greatly reduced by the surface graft polymerization of AAm and HEMA on comparison with the control sample.

21- Cell attachment to the laser-induced AAm and HEMA grafted silicone rubber surface is found to be higher when compared to the CO_2-laser treated PDMS but lower when compared to the control PDMS surface.

22- By increasing the graft density of hydrogels, the topography of the grafted PDMS surface became smooth and has no visible porosity and platelet adhesion on it increases as the graft density increases. We conclude from these results that the morphology, especially the presence of rough surfaces, has a major effect on blood compatibility, which is also observed from the *in vitro* experiments.

23- Comparison of HEMAPC grafted PDMS surfaces with the control, HEMA grafted PDMS and AAm grafted PDMS materials indicate that the platelet adhesion on HEMAPC grafted PDMS is much less than on the other surfaces.

24- HEMAPC grafted PDMS surfaces and ungrafted CO_2-pulsed laser treated PDMS surfaces have better results for blood compatibility than the other treatments. These two surfaces do not induce platelet activation and the number of platelets attached are fewer than for the control and HEMA and AAm grafted PDMS samples.

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ABBREVIATIONS

AAm	**Acrylamide**
Ar	**Argon**
ASTM	**American standard method**
ATR-FTIR	**Attenuated total reflectance Fourier Transform infra red**
AV	**Arterio-venous**
BHK	**Baby Hamster Kidney**
C	**Carbon**
CAB	**Cellulose acetate-butyrate**
CO2	**Carbon dioxide**
CW	**Continuous wave**
DCE	**1,2-dichloroethane**
DCP	**Dicumyl peroxide**
DEE	**Diethyl ether**
DMAEM	**Dimethylaminoethylmethacrylate**
DMTA	**Dynamic mechanical thermal analysis**
DPPH	**1,1-diphenyl-2-picrylhydrazyl**
EB	**Electron beam**
EDTA	**Ethylene diglycol tetraacetic acid**
EDXA	**Energy dispersive x-ray analysis**
EGTA	**ethylene glycol tetraacetic**

EPR	**Ethylene-propylene-rubber**
eV	**Electron volt**
EVA	**Ethylenevinylalcohol**
F	**Fluorine**
FWHM	**Full width at half maximum**
h	**hour**
HEMA	**Hydroxyethylmethacrylate**
HEMAPC	**Hydroxyethylmethacrylate phosphatidyl choline**
IB	**Ion beam**
IRMP	**Infrared multiphoton**
J	**Joule**
KPa	**Kilo Pascal**
KrF	**Krypton Fluoride**
LDPE	**Low density polyethylene**
LDH	**Lactate dehydrogenase**
MMA	**Methylmethacrylate**
MP	**Multiphoton**
MPD	**Multiphoton dissociation**
MPE	**Multiphoton excitation**
MW	**Microwave**
nm	**Nanometre**
NVP	**N-vinyl pyrrolidone**

PAAm	**Poly(acrylamide)**
PBS	**Phosphate buffered saline**
PC	**Polycarbonate**
PDMS	**Polydimethylsiloxane**
PE	**Polyethylene**
PEEK	**Poly(ethyletherketone)**
PET	**Polyethyleneterphthalate**
PHEMA	**Polyhydroxyethylmethacrylate**
Phr	**Per hundred rubber**
PMMA	**Polymethylmethacrylate**
PMMU	**Polymethylmethacrylateurethane**
PP	**Polyethylene**
PPP	**Platelet poor plasma**
PRP	**Platelet rich plasma**
PS	**Polystyrene**
PTFE	**Polytetrafluroethylene**
PVA	**Polyvinylalcohol**
PVC	**Polyvinylchloride**
PVF	**Polyvinylfluride**
PU	**Polyurethane**
QC	**Quasi-continuum**
SEM	**Scanning electron microscopy**
TAH	**Total artificial hips**

TEA	**Transfer excitation at atmospheric pressure**
THF	**Tetra hydrofuran**
TMMS	**Trimethyl(methoxy)silane**
UHMWPE	**Ultr ahigh moleculare weight polyethylene**
UV	**Ultra violet**
VC	**Vinyl chloride**