UDK 533.9:678.7
Original scientific article/Izvirni znanstveni članek

ISSN 1580-2949
MTAEC9, 46(1)63(2012)

THE PLASMA POLYMERISATION PROCESS FOR THE DEPOSITION OF AMINO-CONTAINING FILM ON THE POLY(ETHYLENE TEREPHTHALATE) DRESSING-LAYER FOR SAFE WOUND-HEALING

PLAZEMSKA DEPOZICIJA AMINO-FUNKCIONALIZIRANEGA FILMA NA POLIETILEN TEREFTALATNEM SLOJU OBLOGE ZA UČINKOVITO CELJENJE RAN

Zdenka Peršin[1,2], Adolf Jesih[2,3], Karin Stana-Kleinschek[1,2]

[1]University of Maribor, Faculty of Mechanical Engineering, Laboratory for Characterisation and Processing of Polymers, Smetanova 17, SI-2000 Maribor, Slovenia
[2]Centre of Excellence for Polymer Materials and Technologies, Tehnološki park 24, SI-1000 Ljubljana, Slovenia
[3]Jožef Stefan Institute, Jamova cesta 39, SI-1000 Ljubljana, Slovenia
zdenka.persin@uni-mb.si

Prejem rokopisa – received: 2010-09-28; sprejem za objavo – accepted for publication: 2010-10-10

This article presents a new approach for preparing antimicrobial layer as a part of multi-composite dressing for safe and efficient wound – healing within a moist environment. Plasma polymerisation using a mixture of argon, ammonia, and hexane gases was used for preparing a thin polymer film on the poly(ethylene terephthalate) (PET) surface. The plasma deposition efficiency, regarding the amount of nitrogen, was evaluated by the Kjeldahl method, whilst the absorption of C.I. Acid Orange 7 dye onto accessible amino groups was monitored within the UV/VIS spectral region. The quantitative amount of charged surface groups was determined by potencometric titration.
The results obtained using Kjeldahl method indicated the presence of a substantial amount of nitrogen within the deposited film. Furthermore, mono – azo acidic dye was absorbed onto the polymerised sample, pointing to the formation of an ionic bond between the sulphuric and amino groups. The plasma deposited PET samples resulted in inhibitions regarding all the pathogen microorganisms used, mostly those present in the infected wound.

Keywords: plasma polymerisation, argon, ammonia, hexane, poly(ethylene terephthalate), antimicrobial properties, wound dressing

Prispevek predstavlja nov pristop priprave protimikrobnega sloja, ki ga bo mogoče vključiti v obstoječe ali nove razvite obloge za učinkovito celjenje ran v vlažnem okolju. Postopek plazemske polimerizacije z uporabo mešanice plinov argona, amonijaka in heksana je bil uporabljen z namenom vpeljave funkcionalnih skupin na površino polietilen tereftalata (PET). Učinek nanosa oziroma vsebnost dušika se je ovrednotila s pomočjo konvencionalne Kjeldahl metode. Adsorpcija C.I. Acid Orange 7 barvila na dostopne aminske skupine funkcionaliziranega materiala se je spremljala s pomočjo UV/VIS spektroskopije. Kvantitativna količina funkcionalnih skupin z nabojem se je ovrednotila s potenciometrično titracijo.
Rezultati po Kjeldahl-u nakazujejo na prisotnost dušika v nastalem polimernem filmu na površini PET. Mono – azo barvilo se je uspešno adsorbiralo na površino polimeriziranega vzorca, kar nakazuje na nastanek ionske vezi med sulfonskimi in aminskimi skupinami. Plazemsko polimeriziran polietilen tereftalatni vzorec izkazuje učinkovito inhibicijo na vse testirane bakterijske kulture, ki so največkrat prisotne v okuženi rani.

Ključne besede: plazemska polimerizacija, argon, amonijak, heksan, polietilen tereftalat, protimikrobne lastnosti, obloga

1 INTRODUCTION

Dressing is designed to come into direct contact with a wound. Its properties should meet a number of requirements e.g. to abate blood – flow, to enhance the healing process, to absorb any fluids discharged from the wound, etc. Whilst the wound itself may be of particular cause for concern, the potential infections that could enter the body through the wound opening may be more serious[1].

One important aspect of a dressing usefulness is its ability to prevent infection. The development of such a dressing is thus based either on biologically – active polymers[2] or on the inclusion of potential antimicrobial compounds[3–5] within the dressings. Antimicrobial dressings can be made by binding drug onto the polymers[6–7] or chemically modifying the polymers[8–9] by introducing various functional groups such as amino[10–11], hydroxyl, carboxyl, aldehyde[2,4,12], and combinations of carboxyl and amino, epoxy, methoxy, thiol and sulphone.

There are a wide – variety of dressings possessing antiseptic chemical agents[10,13,14] that have been identified as acting destructively towards microbes, but only a few of them are safe for patients[15–16] and the environment. Their efficiency often decreases after incorporation into a dressing. In addition there is a well – known evidence for bacterial resistance to silver[17–23] that has a long history as an antimicrobial agent.

Plasma polymerization is known as an effective method for the modification of polymer surfaces[24–25] by generating a thin layer of polymerized material containing functional groups. Plasma polymerisation effect is mainly limited in regard to improving the adhesion,

mechanical, and optical properties[26] of those materials mainly developed for usage in agricultural, food and packaging industries, and protective clothing. The deposition of antimicrobial coating through plasma polymerization onto surfaces is mainly focused on improving polymer functional performance and properties[27–28], and the bacterial adhesion and biocompatibility of the biomedical implants[29–30]. Synthetic polymers (e.g. polyethylene terephthalate, polyamide, polypropylene, polyurethane, etc.) are generally applied for packaging and synthesis of textile fibres, whereas their application as medical polymers[31–33], especially as wound dressings, is less known and not exploited much, as yet[34].

Other possible methods for incorporation of nitrogen – rich functional groups into the surface films of polymer materials are functionalization by reactive nitrogen plasma and ion implantation of nitrogen ions[35–37]. The first one employs non – equilibrium plasma created either in pure nitrogen or a mixture of nitrogen and hydrogen or a mixture of nitrogen and a noble gas or ammonia. All these plasmas produce substantial quantities of reactive nitrogen particles[38–39]. Electrodeless discharges often create plasma with a huge density of neutral nitrogen atoms and negligible kinetic energy of nitrogen ions. Capacitively coupled high frequency discharges and simple DC discharges create plasma rich in ions that are accelerated in sheath to relatively high kinetic energies. The concentration of neutral atoms in such discharges is generally smaller than in electrodeless discharges. A best source of energetic ions is a simple ion gun or a more sophisticated ion implantation device. Ion guns create positively charged nitrogen ions with a kinetic energy of the order of 100 or 1000 eV, while implantation devices often operate at much higher kinetic energies[40]. The thickness of the modified film obviously depends on the type of plasma particles and kinetic energy of ions. The surface layer of polymer is enriched with nitrogen using electrodeless discharges. Discharges of reasonable high voltages would cause modification of surface films of the order of few nanometers. Thicker layers are modified using energetic ions either from ion guns or implantation devices.

Baring this in mind, plasma polymerization, using a mixture of argon, ammonia, and hexane gases, was introduced in order to gain antimicrobial activity on the poly(ethylene terephthalate) (PET) surface. A plasma deposited PET surface was characterized by the conventional Kjeldahl method, UV/VIS adsorption studies and potenciometric titrations. The antimicrobial activities of the non-treated and plasma polymerised samples were determined by the AATCC 100-1999 standard test.

In this way modified PET material would be designed to come into direct contact with a wound as a first layer within a multi-layered medical dressing possessing effecting inhibition strategy directed towards skin microorganisms.

2 EXPERIMENTAL PART

2.1 Materials

Poly(ethylene terephthalate) (PET) was studied in its mesh form, as produced by BETI d.o.o., Slovenia. The sample was made of 100 % polyester, with a specific surface mass of 75 g/m^2, and atlas weaving.

2.2 Treatment procedures

Plasma polymerization was carried out in a stainless steel reactor. Stainless steel reactor was an in house constructed modified GEC reference cell[41] of 200 mm i.d. and 284 mm in height. The plasma in the reactor was inductively coupled through a silica window by a five-turn planar coil of 3 mm diameter[42] with a maximum power of 30 W, and at a frequency of 13.5 MHz. During polymerisation the pressure in the reactor was fixed at 0.4 mbar. The sample was placed in a reactor chamber and evacuated to 10^{-3} Pa. When the pressure in the plasma reactor fell below 0.001 mbar, argon gas with a flow rate of 8.0 mL/min (standard conditions, T=0 °C, 1.01 bar) was introduced into the system using a mass flow controller. The material was first treated with argon plasma in order to ensure better adhesion of the plasma polymer to the substrate material. The plasma was ignited with an electric spark and after three minutes, hexane (0.65 mL/min) and ammonia (74.5 % NH_3 in hexane; 0.5 to 6.0 mL/min) were introduced as working gases. The polymerisation procedure lasted 2 h and then the sample was stored in argon atmosphere. Details of the apparatus and treatment procedure are given elsewhere[43].

2.3 Methods

2.3.1 Kjeldahl method

The quantitative amount of nitrogen (%) present in the samples was determined using the conventional Kjeldahl analysis[44]. About 0.5 g of the sample was digested with H_2S0_4 and a catalyst containing 2.8% TiO_2, 3.0% $CuS0_4$ $5H_2O$, and 94.2 % K_2SO_4. The residue was treated with NaOH to liberate NH_3 which was subsequently absorbed in boric acid and titrated with HCl. The total of bound – nitrogen (TN) was determined, in such way that the bound nitrogen was oxidized and thermally – decomposed into NO_2, which was then detected using an electrochemical detector (ChD). The nitrogen oxides underwent oxidation at the anode, causing a change in the current between the electrodes. This change was proportional to the concentrations of nitrogen oxides. All samples were analysed in at least triplicate to ensure reproducibility and to exclude statistical errors.

2.3.2 UV/VIS spectroscopy

The absorption of C.I. Acid Orange 7 (AO7) dye onto the accessible amino groups was monitored using

UV/VIS spectroscopy. Adsorption experiments were carried out at 20 °C, in magnetically – stirred thermostated cylindrical glass vessels, under batch conditions. 2 mL of AO7 dye solution (1.75 g/L) was added to 250 mL of aqueous solution. The pH of the solution was adjusted to 3.66 using Acetic acid. An absorbance value at 482 nm was used to monitor the adsorption process, and the colour was measured according to Lambert–Beer's Law[45] using a UV-Visible Spectrophotometer Cary 50 Conc. The initial absorbance (A_o) of the solution (without a sample) was measured at the beginning, whilst when adding the sample (0.25 g), the absorbance (A_t) was automatically measured as a function of time. The data was collected every 30 s within the first hour, then every hour within 24 h until equilibrium was established. All the experiments were carried out in triplicate. The dye concentration on the sample in equilibrium was calculated as:

$$\frac{c_t}{c_{eq}} = \frac{A_0 - A_t}{A_0 - A_{eq}} \quad (1)$$

where A_o is the initial absorbance, A_t is absorbance in time t, A_{eq} is absorbance in equilibrium, c_t is concentration in time t, c_{eq} is concentration in equilibrium.

2.3.3 Potenciometric titration

Potenciometric titration was used to define quantitative amount of charged groups present in the sample. Potenciometric titration is an electrochemical titration based on determining the volume of the reagent (titrant) that is stehiometrically equal to the amount of measured substance. The pH potentiometric titration of the sample suspension was carried out with a Mettler Toledo T70 two-burette instrument, within an inert atmosphere (N_2 bubbling). The burettes were filled with 0.1 M HCl and 0.1 M KOH. All solutions were prepared in Mili-Q water with low carbonate content (< 10^{-5} M). This was achieved by boiling and cooling in nitrogen atmosphere. The suspension was titrated in a back and forth manner between the initial pH = 2.8 to the pH = 11. The titration experiments were carried out at 0.01 M ionic strength, set to its appropriate value with KCl. The titrant was added dynamically within a step interval of [0.001 – 0.25] mL. The equilibrium criteria was obtained by dE/dt = 0.1/150 s. 150 s was the minimum time to reach equilibrium conditions between the two additions of the titrant, and the maximum time was set at 7200 s. The pH value was measured with a Mettler Toledo DG-117 – combined glass electrode. All the experiments were carried out in triplicate. Blank HCl-KOH titration was carried out under the same conditions as above.

2.3.4 Antimicrobial activity

The general method described in 'AATCC Test Method 100 – 1999, Antibacterial Finishes on Textile Materials: Assessment of'[46] with modifications, was the basis for the protocol used when measuring the qualitative and quantitative antibacterial tendencies of the investigated materials. The three challenging bacterial species were used throughout: *Staphylococcus aureus*, *Escherichia coli*, and *Enterococcus faecalis*. Before each assay, the test bacteria were incubated in either a trypticase soy broth (TSB, BBL® No. 11768 trypticase soy broth) or on trypticase soy agar slants (TSA, BBL® No. 11768 trypticase soy broth, and 2.0% agar) at 37 ± 2 °C for 1 – 3 days, before being used to inoculate the broth's (TSB) cultures for testing. The inoculated broth cultures were incubated at 37 ± 2 °C and stored at 5 ± 1 °C. Standardized density of bacteria ((1 – 2) × 10^5 CFU/mL) was used for the challenge inoculation. For each sample replicate, 1.0 ± 0.1 mL of inoculum was dispersed over the samples, inoculated at 37 ± 2 °C for 24 h, before being assayed for bacterial population density, or were immediately assayed for bacterial population density as the zero – time population density. The bacterial population densities were determined by first extracting the bacteria from the sample by adding 100 mL of diluent to each jar, and then shaking the jars on a table top shaker for 1 min. Then the aliquots were removed and plated directly into petri dishes or further diluted, before being plated. The percentage reduction of bacteria by the samples was determined as:

$$R = 100\ (B–A)/B \quad (2)$$

where R is the reduction (%), A is the number of bacteria recovered from the inoculated test specimen swatches in the jar, incubated over the desired contact period (24 h), and B is the number of bacteria recovered from inoculated test specimen swatches in the jar immediately after inoculation ("zero" contact time).

No antibiotics were used and incubation was at 37 ± 2 °C for at least 24 h before counting the plates.

3 RESULTS

In **Table 1**, the results for the amount of nitrogen per mass of sample (Ni) are shown.

Table 1: Amount of nitrogen per mass (Ni) of non-treated and plasma polymerised samples
Tabela 1: Vsebnost dušika glede na maso (Ni) neobdelanega in plazma polimeriziranega vzorca

Sample	Volume of sample (mL)	Mass of sample (g)	Ni (mmol/kg)
Non-treated	250	0.5133	15.48
Plasma polymerised	250	0.5174	38.62

The Total Nitrogen results indicated a significant increase in the nitrogen present in the deposited film on the PET samples. These results were supported by the infra – red spectroscopy results, determined previously; for details see Z. Persin et al.[47]. The spectra showed an appearance of peaks within 3500 – 3300 cm^{-1}, or at around 1600 cm^{-1}, 1100 cm^{-1}, 900 cm^{-1}, and 700 cm^{-1}.

These peaks are characteristic for R-NH_2 functional groups.

The concentrations of the adsorbed C.I. Acid Orange 7 dye on the samples, depending on the treatment are presented in **Table 2**.

Table 2: Concentrations of the adsorbed C.I. Acid Orange 7 dye on the non-treated and plasma polymerised samples
Tabela 2: Koncentracija barvila C.I. Acid Orange 7 na neobdelanih in plazemsko polimeriziranih vzorcih

Sample	Concentration of AO7 dye on the sample (mmol/kg)	Concentration of AO7 dye on the sample (g/g)
Non-treated	0.00368	1.3 E-06
Plasma polymerised	0.16607	5.8 E-05

A very low concentration of AO7 dye was adsorbed on the non-treated sample, whilst the dye concentration on plasma – polymerised sample was significantly higher.

Table 3 present the amount of charged groups in the non-treated and plasma-polymerised poly(ethylene terephthalate) samples.

Table 3: Amount of charged groups in the non – treated and plasma polymerised poly(ethylene terephthalate) samples
Tabela 3: Količina funkcionalnih skupin z nabojem v neobdelanem in plazemsko polimeriziranem polietilen tereftalatnem materialu

Sample treatment	Amount of charged groups (mmol/kg)	
	Positively	Negatively
Non-treated	12.7	–
Plasma polymerised	19.4	–

Both PET samples, regardless of the treatment used, exhibited no negative charge, whilst the non-treated PET sample showed approximately 13 mmol/kg of positive charge. The PET mesh coated with a plasma-polymerized film revealed 19.4 mmol/kg of positive charge. Compared to the non-treated sample, the treated sample showed an increase of positive charge for 6.4 mmol/kg.

The results for antimicrobial activity by non-treated and plasma polymerised PET samples are presented in **Table 4**. The results represent a reduction (*R*) of bacteria that are likely to be present in an infected wound.

Table 4: Reduction R (%) of the bacteria, mostly present in the infected wound, for non-treated and plasma polymerised sample
Tabela 4: Stopnja redukcije R (%) neobdelanega in plazemsko polimeriziranega materiala na bakterije, ki so najpogosteje prisotne v okuženi rani

Sample	Reduction *R* (%) on bacterial culture		
	Staphylococcus aureus (gram positive)	*Escherichia coli* (gram negative)	*Enterococcus faecalis* (gram positive)
Non-treated	75 %	No reduction	No reduction
Plasma polymerised	100 %	99.96 %	93.7 %

The results for the non-treated samples, as shown in **Table 4**, indicate no reduction for *Escherichia coli* and *Enterococcus faecalis*, but a significant reduction for 75% for *Staphylococcus aureus.* The plasma polymerised PET samples indicated higher antimicrobial activity. The deposited film containing amino groups resulted in inhibition on all the used pathogen microorganisms. These results are valid within the limits of the experimental error.

4 DISCUSSION

A polymer film containing nitrogen functional groups arose on the material surface after applied plasma polymerisation. The efficiency of plasma deposition resulted in a 150 % increase of total nitrogen content as revealed from measured IR – spectra.

UV/VIS spectrophotometric results indicate increased concentrations of AO7 dye on the plasma polymerised samples. The groups introduced by plasma polymerisation present accessible locations for the efficient binding of mono – azo acidic dye molecules. The ionic bond between dye sulphate groups (SO_3^-) and amino (NH_3^+) groups introduced by plasma polymerisation occur in a stehiometric ratio of 1 to 1.

Results obtained by potenciometric titration indicate by non-treated sample a presence of a positive charge groups. This surprisingly existence of positive charge might be explained due to dyes and other chemicals used during the manufacturing process of the PET sample in a form of a mesh. The PET sample coated with a film deposited by plasma polymerization shows a higher amount of positive charge, which could be attributed to the introduction of amino groups by plasma polymerisation treatment. On contrary, no negative charge by non – treated sample as well by plasma polymerised samples was observed. The obtained results could be explained due to fact that titration is a technique used for determining the H^+ ions resulting due to present functional groups that are able to dissociate (e.g. weak acid, amino groups). Since basic PET molecule do not possess weak acid functional groups, while plasma treated sample possess amino groups, the obtained results are reasonable and explain the discrepancy regarding high negative surface charge groups obtained in the same material by other authors[48–49].

When compared with the non-treated sample, only a 75 % reduction was evidenced on *S. aureus*. This result was rather unpredictable, although the percentage does not account for antimicrobial activity; the noticeable antimicrobial challenge delivering a higher value[50]. In addition, it could indicate that the inhibition of the specific bacteria is not only due to the presence of amino groups, but could also be due to the changed (i.e. improved) hydrophilic properties of the tested material[51]. In addition, bacteria exist over a wide range of shapes, ranging from spheres to rods and spirals, which differ in dimensions. *S. aureus* is a round – shaped cocci bacterium with a diameter of 0.5 – 1.0 μm[52], compared to the

rod – shaped *E. coli* that is 0.5 – 1.0 μm wide and 1 – 4 μm long[53]. In this agreement, the bacteria molecule having smaller diameter could more easily penetrate into the porous sample, resulting in apparent antimicrobial activity.

A PET material coated with a layer deposited by plasma polymerisation turned out to be antimicrobial. Such a property is expressed for all the tested bacteria. Within the limits of experimental error a 100 % reduction was observed for *Staphylococcus aureus*, which is one of the more important pathogens, causing illnesses from minor skin infections to life – threatening diseases. Its incidences are from skin, soft tissue, respiratory, bone, joint, and endovascular wound infections. *S. aureus* is nowadays one of the five most common causes of nosocomial infections, often causing postsurgical wound infections. A 93.7 % reduction was obtained for the second gram – positive bacteria used. *E. faecal*[54] is listed as the first to the third leading cause of nosocomial infections. Most of these infections occur after surgery on the abdomen or a puncturing trauma, but can also be linked to the increased use of catheters, which are considered compromising devises. It is also responsible for urinary tract infections, bacterimia, endocarditis, meningitis, and can be found in wound infections along with many other bacteria[54]. A noteworthy reduction (99.96 %) was also evident for gram – negative bacteria from a species type of the genus Escherichia. The *E. coli* bacteria is known for spreading from one person to another, as well as being able to spread from an infected person hands to other people or to objects[55].

5 CONCLUSION

The plasma polymerization process proved to be a successful tool for polymer surface functionalization. Using the proposed preparation procedure, a universal layer could be prepared and incorporated into newly – prepared and also existing multilayer composites. Based on the inertness and acquired antimicrobial activity of such a layer, it could even be used for dressings having direct contact with the wound. The latter enables the use of such dressings, even for patients suffering from hypersensitive reactions.

Acknowledgement

This work was supported by the Ministry of Higher Education, Science and Technology of the Republic of Slovenia [Grant number 3211-10-000057].

6 REFERENCES

[1] T. Mustoe, American Journal of Surgery, 187 (**2004**) 5A, 65S–70S
[2] I. A. Chekmareva, Bulletin of Experimental Biology and Medicine, 133 (**2002**), 226–30
[3] A. R. Lee, W. H. Huang, Journal of Pharmacy and Pharmacology, 47 (**1995**) 6, 503–9
[4] J. Hart, D. Silcock, S Gunnigle, International Journal of Biochemistry & Cell Biology, 34 (**2002**), 1557–70
[5] A. E. C. C. Pecharroma´n, E.A. J. S. S. Moya, Journal of Materials Science, 41 (**2006**), 5208–5212
[6] C. Watanakunakorn, C. Glotzbecker, Antimicrobial Agents and Chemotherapy, 11 (**1977**) 1, 88–91
[7] M. R Wessels, Streptococcal and enterococcal infections, in Harrison's principles of internal medicine, 14th edition, (Isselbacher, K. J., Braunwald, E., Wilson, J. D., Martin, J. B., Fauci, A. S., Kasper, D. L., eds), McGraw-Hill, Inc (Health Professions Division), 1998, 890–891
[8] T. N. Yudanova, I. V. Reshetov, Pharmaceutical-Chemistry-Journal, 40 (**2006**), 24–31
[9] L. G. Ovington, Clinics in Dermatology, 25 (**2007**), 33–8
[10] P. Gilbert, L. E. Moore, Journal of Applied Microbiology, 99 (**2005**) 4, 703–15
[11] L. Zemljic Fras, S. Strnad, O. Sauperl, Textile Research Journal, 79/3 (**2009**), 219–226
[12] K. Jansson, A. Haegerstrand, G. Kratz, Scandinavian Journal of Plastic and Reconstructive Surgery and Hand Surgery, 35 (**2001**), 369–75
[13] J. B. Wright, K. Lam, D. Hansen, R. E. Burrell, American Journal of Infection Control, 27 (**1999**) 4, 344–50
[14] H. Q. Yin, R. Langford, R. E. Burrell, Journal of Burn Care & Rehabilitation, 20 (**1999**) 3, 195–200
[15] J. F. Fraser, L. Cuttle, M. Kempf, R. M. Kimble, ANZ Journal of Surgery, 74 (**2004**) 3, 139–42
[16] S. L. Percival, P. G. Bowler, D. Russell, Journal of Hospital Infection, 60 (**2005**) 1, 1–7
[17] G. L. McHugh, R. C. Moellering, C. C. Hopkins, M. N. Swartz, Lancet, 1(7901) (**1975**), 235–40
[18] R. P. Wenzel, K. J. Hunting, C. A. Osterman, M. A. Sande, American Journal of Epidemiology, 104 (**1976**) 2, 170–80
[19] W. E. Gayle, C. G. Mayhall, V. A. Lamb, E. Apollo, B. W. Haynes, Journal of Trauma-Injury Infection and Critical Care, 18 (**1978**) 5, 317–23
[20] K. Bridges, A. Kidson, E. J. Lowbury, M. D. Wilkins, British Medical Journal, 1 (**1979**) 6161, 446-9
[21] X. Z. Li, H. Nikaido, K. E. Williams, Journal of Bacteriology, 179 (**1997**) 19, 6127–32
[22] H. J. Klasen, Burns, 26 (**2000**) 2, 131–8
[23] N. McCallum, B. Berger-Bächi, M. M. Senn, International Journal of Medical Microbiology, 300 (**2010**) 2–3, 118–129
[24] H. Yasuda, Journal of Polymer Science Macromolecular Reviews, 16 (**1981**), 199–293
[25] H. Yasuda, Journal of Membrane Science, 18 (**1984**), 273–284
[26] E. M. Bachari, S. Ben Amora, G. Baud, M. Jacquet, Materials Science and Engineering B-Solid State Materials for Advanced Technology, 79 (**2001**) 2, 165–174
[27] K. H. Hong, N. Liu, G. Sun, European Polymer Journal, 45 (**2009**) 8, 2443–2449
[28] R. Davis, A. El-Shafei, P. Hauser, Surface & Coatings Technology, 205/20 (**2011**) 15, 4791–4797
[29] J. Wanga, N. Huang, P. Yang, Y. X. Leng, H. Sun, Z. Y. Liu, P. K. Chu, Biomaterials, 25/16 (**2004**), 3163–3170
[30] S. Lerouge, A. Major, P. L. Girault-Lauriault, M. A. Raymond, P. Laplante, G. Soulez, F. Mwale, M. R. Wertheimer, M. J. Hébert, Biomaterials, 28/6 (**2007**), 1209–1217
[31] W. Zhang, J. Jia, Y. Zhang, Q. Yan, E. Z. Kurmaev, A. Moewes, J. Zhao, P. K. Chu, Applied Surface Science, 253/22 (**2007**), 8981–8985
[32] T. P. Martin, S. E. Kooi, S. H. Chang, K. L. Sedransk, K. K. Gleason, Biomaterials, 28/6 (**2007**), 909–915
[33] J. Wang, J. Li, L. Ren, A. Zhao, P. Li, Y. Leng, H. Sun, N. Huang, Surface & Coatings Technology, 201/15 (**2007**), 6893–6896

[34] D. Hegemann, M. Mokbul Hossain, D. J. Balazs, Progress in Organic Coatings, 58 (**2007**), 237–240
[35] A. Vesel, I. Junkar, U. Cvelbar, J. Kovac, M. Mozetic, Surf. Interface Anal., 40/11 (**2008**), 1444–1453
[36] A. Vesel, M. Mozetic, S. Strnad, Vacuum, 85/12 (**2011**), 1083–1086
[37] A. Vesel, M. Mozetic, A. Zalar, Surf. Interface Anal., 40 (**2008**) 3–4, 661–663
[38] M. Mozetic, U. Cvelbar, A. Vesel, A. Ricard, D. Babic, I. Poberaj, J. Appl. Phys., 97 (**2005**) 10, 103308-1–103308-7
[39] F. Gaboriau, U. Cvelbar, M. Mozetič, A. Erradi, B. Roufflet, J. Phys. D Appl. Phys., 42 (**2009**) 5, 055204-1–055205-5
[40] J. Jagielski, G. Gawlik, A. Zalar, M. Mozetič, Inf. MIDEM, 29 (**1999**) 2, 61–67
[11] P. J. Hargis Jr., K. E. Greenberg, P. A. Miller, J. B. Gerardo, J. R. Torzynski, M. E. Riley, G. A. Hebner, J. R. Roberts, J. K. Olthoff, J. R. Whetstone, R. J. Van Brunt, M. A. Sobolewski, H. M. Anderson, M. P. Splichal, J. L. Mock, P. Bletzinger, A. Garscadden, R. A. Gottscho, G. Selwyn, M. Dalvie, J. E. Heidenreich, J. W. Butterbaugh, M. L. Brake, M. L. Passow, J. Pender, A. Lujan, M. E. Elta, D. B. Graves, H. H. Sawin, M. J. Kushner, J. T. Verdeyen, R. Horwath, T. R. Turner, Rev. Sci. Instrum., 65 (**1994**), 140–154
[42] P. A. Miller, G. A. Hebner, K. E. Greenberg, P. D. Pochan, B. P. Aragon, Res. Natl. Inst. Stand. Technol., 100 (**1995**), 427–439
[43] P. Klampfer, T. Skapin, B. Kralj, D. Žigon, A. Jesih, Acta Chimica Slovenica, 50 (**2003**), 29–41
[44] K. Vaideki, S. Jayakumar, R. Rajendran, G. Thilagavathi, Applied Surface Science, 254 (**2008**), 2472–2478
[45] P Atkins in J. de Paula, Atkins' Physical Chemistry, 7th Edition, Oxford University press Inc., New York 2002, 483–497
[46] Anonymous, 1999 Anonymous, 'AATCC Test Method 100-1999, Antibacterial finishes on textile materials: Assessment of' AATCC Technical Manual 75 (2000), Technical Manual of the American Association of Textile Chemists and Colorists 1999, AATCC, Triangle Park, NC 1999, 147–149
[47] Z. Peršin, K. Stana-Kleinschek, A. Jesih, U. Maver, 4th International Conference on Advanced Plasma Technologies (iCAPT-IV) with Workshop on Plasma Synthesis and Applications of Nanomaterials & 112th IUVSTA Executive Council Meeting, September 9th [i. e.] 11th – September 13th 2011, Strunjan, Slovenia, Conference proceedings. Ljubljana: Slovenian Society for Vacuum Technique: = DVTS – Društvo za vakuumsko tehniko Slovenije, (2011), 127–130
[48] T. Indest, S. Strnad, K. S. Kleinschek, V. Ribitsch, L. Fras, Colloids and Surfaces A: Physicochemical and Engineering Aspects, 275 (**2006**) 1–3, 17–26
[49] J. Wang, N. Huang, P. Yang, Y. X. Leng, H. Sun, Z. Y. Liu, P. K. Chu, Biomaterials, 25 (**2004**) 16, 3163–3170
[50] L. Fras-Zemljic, V. Kokol, D. Cakara, DOI: 10.1177/0040517511404600
[51] L. Fras Zemljic, O. Sauperl, I. But, A. Zabret, M. Lusicky, DOI: 10.1177/0040517510397572
[52] U. Cvelbar, M. Mozetič, N. Hauptman, M. Klanjšek Gunde. J. Appl. Phys., 106 (**2009**) 10, 103303-1–103303-5
[53] K. Elersic, I. Junkar, A. Spes, N. Hauptman, M. Klanjsek Gunde, A. Vesel, Mater. Tehnol., 44 (**2010**) 3, 153–156
[54] K. J. Ryan, C. G. Ray, Sherris Medical Microbiology (4th ed.), McGraw Hill, 2004, 294–5
[55] K. Todar, "Pathogenic E. coli", Online Textbook of bacteriology, University of Wisconsin–Madison Department of Bacteriology, Retrieved 2007, 11–30