Tomas Bata University in Zlín
Faculty of Technology

Doctoral Thesis

The influence of surface properties of materials on biofilm formation

Vliv povrchových vlastností materiálů na tvorbu biofilmu

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ABSTRACT

To apply polymeric materials in either industry or biomedicine it is essential to know not only their material but also biological properties. Conducting polymers (CPs), have become the subject of intensive research thanks to their unique properties, such as conductivity, simple and low-cost synthesis or easy coating of various surfaces by CP. The potential application of CPs has huge diversity, e.g. they can be used for biosensors, neural implants, tissue engineering scaffolds or stimuli-responsive devices. Between the unique properties of CP can be assigned the easy modification either chemically (e.g. by using various doping acids), by plasma treatment or incorporation of antimicrobial agents onto their surface. The ability of easy surface modification is crucial for their application as biointerface materials. Due to these, CPs can be also used for the improvement of the surface properties of other materials. Improved surface properties may subsequently influence the reaction and attachment of various proteins, eukaryotic cells, tissues and especially microorganisms.

The presence of microbial biofilm and overall the adhesion of microorganisms onto the material surfaces cause severe problems in many fields of industry or medicine. In the biofilm community, the cells are able to effectively protect themselves against external conditions and extracellular matrix prevents the penetration of any foreign substances such as biocides, antimicrobial agents (antibiotics, etc.). The surface properties of materials are a key factor influencing the reciprocal interaction between surface and microorganisms, e.g. in the context of microorganism attachment.

Thus, in the continuity of this issue, the main aim of the doctoral study was to provide novel information about the possible anti-biofilm properties of pristine CPs or their modification. The present information can be applied in the design of materials that will subsequently be capable to prevent the adhesion of microorganisms onto their surfaces.

Keywords: *polymeric materials, conducting polymers, films, surface properties, microorganisms, biofilm.*

ABSTRAKT

Uplatnění polymerních materiálů nejen v průmyslu, ale i v biomedicíně vyžaduje znalost jejich materiálových vlastností, především pak vlastností povrchových, ale také vlastností biologických. Elektricky vodivé polymery se staly předmětem výzkumu z důvodu potenciálního využití v mnoha odvětvích průmyslu či biomedicíny. Zájem o vodivé polymery vychází z jejich unikátních vlastností jako je elektrická vodivost, snadná a nízkonákladová syntéza či jednoduchá tvorba tenkých filmů na různých površích. Potenciální využití vodivých polymerů je tak velmi rozmanité a perspektivní, ať už v oblasti biosenzorů, neurálních implantátů, scaffoldů pro tkáňové inženýrství či jako zařízení reagující na stimuly.

Vodivé polymery se vyznačují jednoduchou modifikovatelností jak chemicky, pomocí dopujících kyselin, tak pomocí plazmového ošetření či inkorporací antimikrobiálních látek. Vodivé polymery tak mohou být využity k ovlivnění chování rozličných biologických systémů, jako jsou proteiny, jednotlivé buňky (prokaryotické i eukaryotické) či tkáně. V řadě aplikací jsou pak biofilm tvořící mikroorganismy významnější než častěji studované planktonní kmeny. To je dáno tím, že přítomnost biofilmu způsobuje vážné problémy v mnoha oblastech průmyslu a medicíny. Odstranění již vytvořeného mikrobiálního biofilmu se stává rovněž náročnou záležitostí, jelikož v tomto společenství se buňky dokážou lépe chránit vnějším vlivům a extracelulární matrix zabraňuje penetraci biocidních látek. Povrchové vlastnosti materiálů proto hrají klíčovou roli při interakci materiálu s mikroorganismy, protože mohou ovlivnit již počáteční adhezi mikroorganismů.

V návaznosti na problematiku vzniku a rozvoje biofilmu je hlavním cílem této dizertační práce poskytnout základní informace a poznatky o schopnosti omezit tvorbu bofilmu pomocí vodivých polymerů a jejich modifikací stejně jako pomocí dalších materiálů.

Klíčová slova: *polymerní materiály, vodivé polymery, filmy, povrchové vlastnosti, modifikace, mikroorganizmy, biofilm.*

1 INTRODUCTION

The life on Earth could not exist without naturally occurring biomacromolecules. Their role is various, e.g. to carry the information (nucleic acids), catalysis (enzymes), be structural elements (fiber-forming proteins, cellulose), energy (glycogen, polyester), or transport (e.g. hemoglobin). A man has always used biopolymers as materials – wood, wool, etc. Nowadays, a wide range of naturally occurring biopolymers derived from renewable resources is available for material applications. The application of a few of them is abundantly used for the production of many kinds of products every day (e.g. cellulose and starch). Whereas many other biopolymers remain underutilized. Biopolymers are derived from a diverse set of molecules such as polysaccharides, proteins or lipids which are produced by different species of microorganisms, plants, and animals. Although the biopolymers provide a number of advantageous properties, they also have a number of limitations, e.g. in terms of inherent biodegradability or temperature stability (Kaplan, 1998). Therefore, the production of synthetic polymers has started and their attractive properties contribute to many various fields. Moreover, the synthetic polymers can be subsequently combined together or with biopolymers, to provide a new composite with unique properties. This is very important for example in the context of antimicrobial properties of surfaces.

For modification of polymeric surfaces, a lot of procedures exist, such as plasma treatment, modification using biologically active doping acids, e.g. poly(acrylamidomethylpropanesulphonic acid) (PAMPSA) which is know to influence hemostasis (Ul Ahad *et al.*, 2014; Humpolicek *et al.*, 2015; Bober *et al.*, 2015) or incorporation of antimicrobial agents onto polymeric surface (e.g. antibiotics) (Jain *et al.*, 2014). Incorporation of antimicrobial agents can be achieved through a number of techniques, including physical adsorption, covalent attachment or doping procedure (Guimard, Gomez and Schmidt, 2007). Despite all the modifications, microorganisms continuously evolve the protection against old as well as new antibiotic agents and strategies. The consequences of microbial cell growth have become a far-reaching issue causing not only infections but also unfavorable changes of the surfaces. Material scientists therefore endeavor to develop new antibiofouling agents or strategies to eradicate microbial biofilm.

One group of synthetic polymers which can contribute to antimicrobial materials is a group of conducting polymers (CP). CPs are widely studied for their outstanding properties. They can easily form thin films on other materials and cover their entire area. Moreover, CPs have low-cost production and rapid polymerization process (Anselme, Ploux and Ponche, 2010). However, it still persists the desire to further optimize their surfaces and forms with targeting to specific applications.

CPs, their composites, and mutual combinations are generally studied for application in medicine, biotechnology, nanotechnologies, tissue engineering, sensors, drug delivery or manufacturing (Guo and Ma, 2018; Shah *et al.*, 2018; Ibanez *et al.*, 2018; Zhang, Dong and Hu, 2018; Ghasemi-Mobarakeh *et al.*, 2011; Nambiar and Yeow, 2011; Gomez *et al.*, 2011). The important fact for utilizing CPs in any application is to determine their properties. Concerning the antibacterial, or especially the antibiofilm properties, the surface parameters among which belong to chemical, electrical, thermal or atomic properties are crucial (Rodríguez-Hernández, 2016). It is moreover well-known that the first substances, which are in contact with the colonized surface, may not be microorganisms themselves, but trace organics. However, trace organics form a thin layer neutralizing excessive surface charge and surface free energy. This founding might contribute to knowledge about bacterial attachment onto the surface and prevent the initial bacterial approach. Furthermore, most microorganisms adhere more rapidly to hydrophobic, nonpolar surfaces (Rodney, 2002; De-la-Pinta *et al.*, 2019).

To solve the problem of biofilm formation the scientists endeavor to develop new effective antibiofilm surfaces focused on prevention microbial attachment from the initial contact. Therefore, the subject of this doctoral thesis is to describe preferably the effect of CPs, and further of titanium-based materials, on microbial biofilm formation.

1.1 Polymeric materials

Polymers are materials of the twentieth century. However, the era of synthetic polymers started in the late-nineteenth century, and the synthesis and science of the polymers evolved enormously in the twentieth century (Pious and Thomas, 2016). The development of polymeric materials, as engineering materials, came hand in hand with the results of their extensive research (Ehrenstein, 2001). Thanks to the composition of these materials consisting (in most cases) of carbon and hydrogen atoms, industrial companies started to benefit from their specific characteristics, which are different in comparison to steel materials (Ehrenstein, 2001). Nowadays, polymeric materials are using almost in all areas of daily life. Their production and fabrication represent significant volumes of total industrial worldwide production (Inzelt, 2008; Fried, 2014).

The fact is that polymeric materials are more sensitive to processing and environmental conditions than traditional engineering materials, such as steel. Nevertheless, polymers have significant benefits including a weight-to-strength ratio and the relative ease of processing and component integration. These facts and the basic understanding of their fundamental behavior make them attractive for use in various applications (Ehrenstein, 2001). Although polymers are not as stiff or strong as traditional engineering materials, they may be often compared to metals and ceramics, with respect to low density, stiffness and strength on a per-mass basis. Polymeric materials show relatively acceptable chemical stability and they are non-reactive in a large number of environments (Callister Jr and Rethwisch, 2012). Thus, polymeric materials can even substitute traditional materials in a broad range of applications (Pious and Thomas, 2016). In addition to all the above-mentioned advantages, some of the polymeric materials are also popular thanks to special and useful properties, such as conductivity. This characteristic gave rise to a unique subgroup of polymers – conducting polymers (CPs).

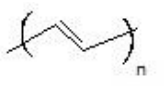
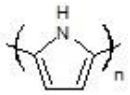
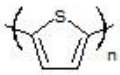
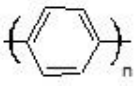
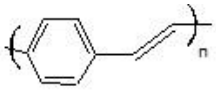
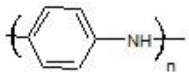
1.1.1 Conducting polymers

CPs, as a novel generation of organic materials, were firstly produced in the 1970s (Shirakawa *et al.*, 1977; Guimard, Gomez and Schmidt, 2007). Interest in CPs arose mainly due to their electrical and optical properties, which are often similar to metals and inorganic semiconductors. Their attractive characteristics associated with properties similar to those of conventional polymers (flexibility in processing, easy of synthesis) are also in the center of interest (Guimard, Gomez and Schmidt, 2007; Le, Kim and Yoon, 2017). One of the major discoveries related to CPs is attributed to the team of H. Shirakawa, who has synthesized the simplest CP, polyacetylene. In 1997, they reported a 10 million-fold increase in the conductivity of polyacetylene doped with iodine compared to silvery films of *trans* polyacetylene without halogenation process (undoped with iodine). Thus, polyacetylene was recognized as the first inherent CP. The

discovery of Shirikawa's team and the development of electronically CPs were awarded by the Chemistry Nobel Prize in 2000 (Rasmussen, 2011). Polyheterocycles, such as polyaniline (PANI), polypyrrole (PPy), polythiophene (PTh), poly(3,4-ethylendioxythiophene) (PEDOT), were further developed in the 1980s. These CPs have emerged as another class of aromatic CPs exhibiting good stabilities, conductivities, and ease of synthesis (Kundu and Giri, 1996; Hong and Marynick, 1992). Nowadays, there is a number of CP systems with advantageous properties applicable in many fields. Thanks to the above-mentioned advantages of CPs, they attract the attention of many researchers (Balint, Cassidy and Cartmell, 2014).

The chemical structure, typical conductivities and year of discovery of some electroactive polymeric materials (EPM) are shown in Tab. 1.

Tab. 1: The chemical structure, typical conductivities and year of discovery of some electroactive polymeric materials. PA: polyacetylene; PPy: polypyrrole; PTh: polythiophene; PPP: poly(p-phenylene); PPV: poly(phenylene vinylene); PANI: polyaniline (modified according to (Jian-xun, Key Laboratory of Polymer and Key Laboratory of Polymer Ecomaterials, 2015; Kumar and Anand, 1998).

EPM	Chemical structure	Conductivity (S cm^{-1})	Discovery year
PA		10^5	1977
PPy		600	1978
PTh		200	1981
PPP		500	1979
PPV		1	1979
PANI		10	1980

However, it would be also appropriate to mention the greatest advantage of CPs, which is versatility (Fig. 1). Thanks to versatility, the CPs may be used in a diverse array of fields, ranging from sensors devices to tissue engineering. The unique property of CPs that links all their applications together is conductivity (Kaur *et al.*, 2015; Liu *et al.*, 2018; Ning *et al.*, 2018).

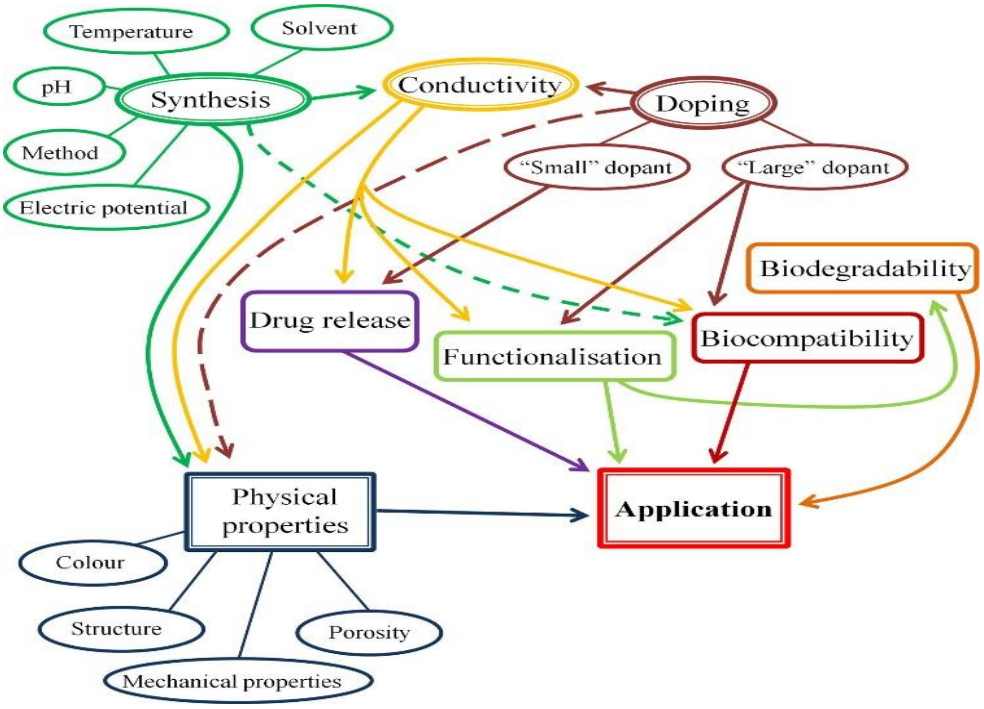


Fig. 1: Everything is connected in the world of conducting polymers.
(Balint, Cassidy and Cartmell, 2014)

The conductivity of CPs is also one of the subjects discussed in this doctoral thesis. The conductivity is correlated with surface properties of CPs, which have subsequently impact on biocompatibility, anti-corrosion or anti-biofouling properties of CPs.

1.1.1.1 Structure and synthesis of conducting polymers

The electrical properties of CPs are predominantly influenced by the structure of their backbone, present functional groups, morphology, and oxidation state. The conjugated structure of their chain consists of alternating single and double bonds or conjugated segments coupled with atoms providing p-orbitals for continuous orbital overlap. These bonds endow the polymer with metal-like semiconductor properties (Fig. 2) (Dai, 2006). Several studies have demonstrated that the planar conformation of the alternating double-bond system is critical for conductivity, as this system maximizes sideway overlap between the π molecular orbitals (Guimard, Gomez and Schmidt, 2007; Kaloni *et al.*, 2017); (Tourillon, 1986).

As regards structure, the simplest CP is polyacetylene, which is also one of the most studied CPs (Szuwarzynski, Wolski and Zapotoczny, 2016). Despite its high conductivity, nowadays, the attention is directed rather to more stable polymers, such as PANI or PPy. The structure of selected CPs is shown in Fig. 3.

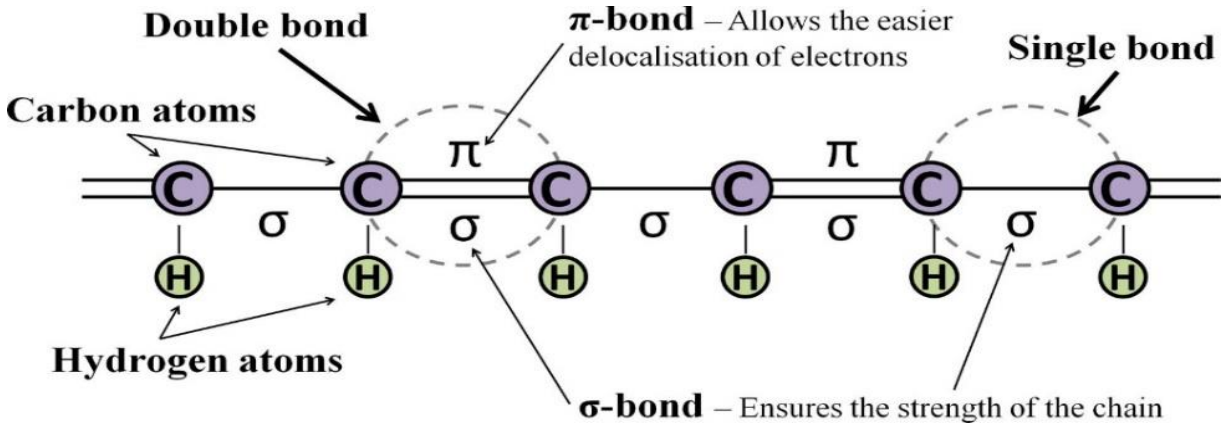


Fig. 2: A simplified schema of a conjugated polymer backbone: a chain containing alternating single and double bonds.
(Balint, Cassidy and Cartmell, 2014)

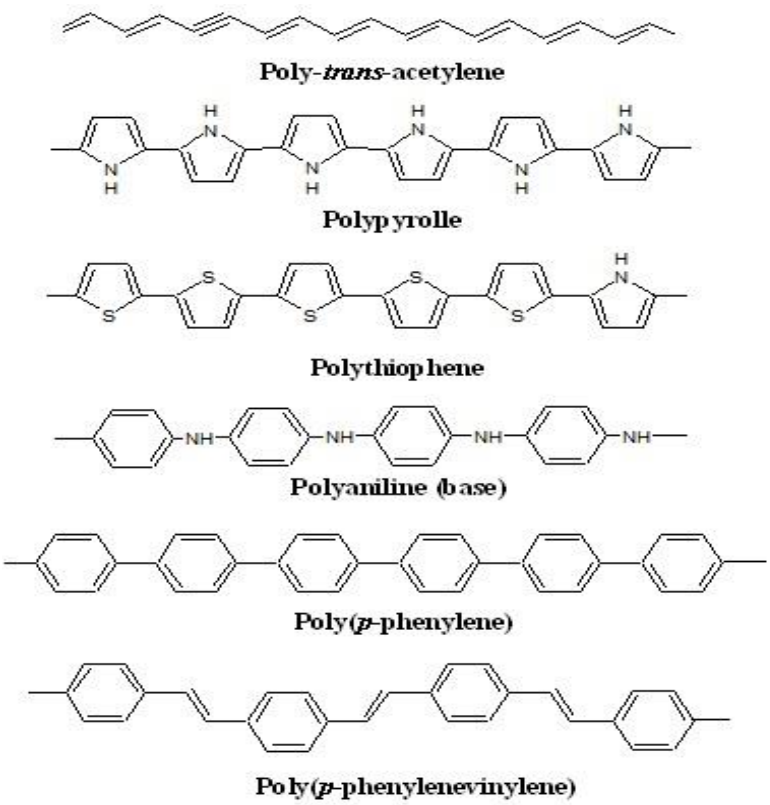


Fig. 3: The structure of selected CPs.
(modified according to Stejskal, 2006)

Except for conjugation, the presence of charge carriers mediating its transport along the chain is the essential factor for high electrical conductivity. The CPs arise from a process called doping. Doping is the process of oxidizing (p-doping) or reducing (n-doping) a neutral polymer. It provides a counter anion or cation such as dopant (Guimard, Gomez and Schmidt, 2007). For inorganic and organic semiconductors, the only trace amount of doping substance influences electrical properties. In the case of CPs, however, the higher dopant concentrations are required to influence their electrical properties (Macdiarmid *et al.*, 1985; MacDiarmid and Epstein, 1995). The conductivity of CPs can be augmented by increasing the doping percentage and varying the dopant type (Guimard, Gomez and Schmidt, 2007). Next to electroactivity, the chemical dopant affects the surface and bulk structural properties of CPs. Small and large dopants (in terms of size of molecule) can modulate both electrical conductivities and surface properties. However, larger dopants can even change material characteristics, such as surface topography and other physical properties (Collier *et al.*, 2000).

CPs are commonly prepared by two different processes, chemical or electrochemical polymerization.

1) Chemical synthesis

CPs are chemically synthesized mainly through the oxidation of monomers (precursors). During this process, the monomer solution is mixed with an oxidizing agent (e.g. ferric chloride, ammonium persulfate) (Tan and Ghandi, 2013). Thereupon, powders or polymer films are formed (Calvo *et al.*, 2002). Thanks to the possibility of the mass production of CPs at a reasonable price, the popularity of chemical synthesis has increased (Kumar, Singh and Yadav, 2015; Sharma, Sims and Mazumder, 2002). Also, PANI or PPy, can be prepared by chemical synthesis. However, electrochemically prepared variants of these CPs have frequently improved conductivity and mechanical properties (Kumar, Singh and Yadav, 2015).

Many studies are focused on the investigation of CPs prepared by the chemical way of synthesis. PANI and PPy are certainly the most frequently studied representatives (Kumar and Yadav, 2016; Abu-Thabit, 2016; Apetrei *et al.*, 2018; Yuan *et al.*, 2016; Kausaite-Minkstimiene *et al.*, 2015) and numeral studies have been applied to increase the yield and quality of the products attained *via* this polymerization process (Veerasubramani *et al.*, 2016; ElNahrawy *et al.*, 2017). The chemical polymerization was used for the preparation of tested PANI samples used within the researches conducted in this doctoral thesis (Fig. 4).

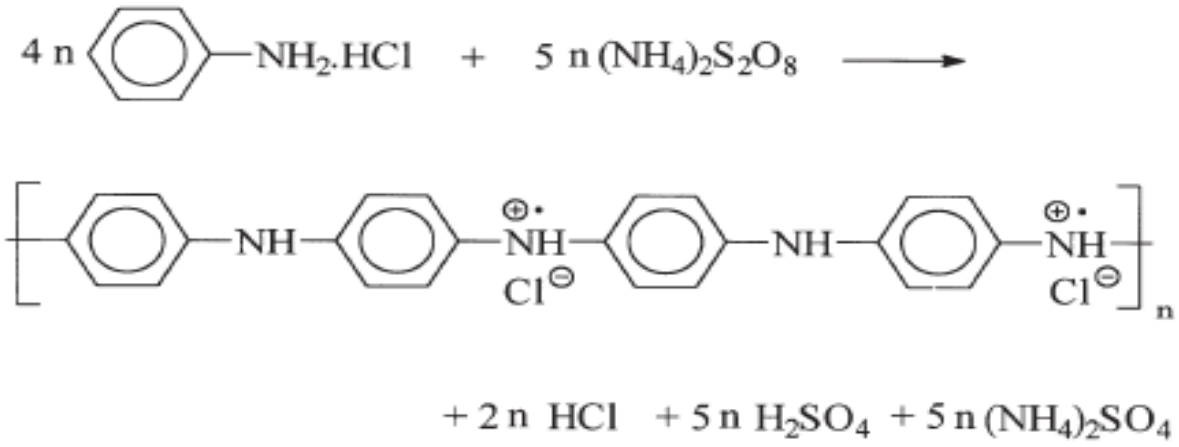


Fig. 4: Chemical synthesis of PANI using the oxidation of aniline hydrochloride with ammonium persulfate. (Stejskal and Gilbert, 2002)

2) Electrochemical synthesis

This type of synthesis is based on the direct oxidation of the monomer in the electrolyte solution by applied electric potential and the growth of the polymer chain on the anode surface (Goto *et al.*, 2008). As already mentioned above, electrochemically prepared CPs own frequently improved conductivity. The electrochemical doping/dedoping of ions is achieved by the redox process in an electrolyte which contains a supporting salt. The reduction process replaces the electron in the polymers and releases the ion. The electrochemical doping/dedoping process gives rise to a change in the electronic band structure of the CP. It is accompanied by a visible color change (Goto *et al.*, 2008). As there is no need to use an oxidizing agent, it is possible to achieve higher purity of the polymer.

The electrochemical synthesis is influenced by many factors, such as the composition of the reaction solution (i.e. electrolyte, temperature), electrode material and electrochemical technique (galvanostatic, potentiostatic and potentiodynamic methods) (Gvozdenovic *et al.*, 2014). This synthesis type offers better control of thickness, morphology, and degree of doping of a polymer film. Nevertheless, the disadvantage is a limited sample size, which is predetermined by the size of the electrode in a cell (Kaynak and Foitzik, 2010). Figure 5 shows the set-up schema of the electrochemical synthesis of CPs.

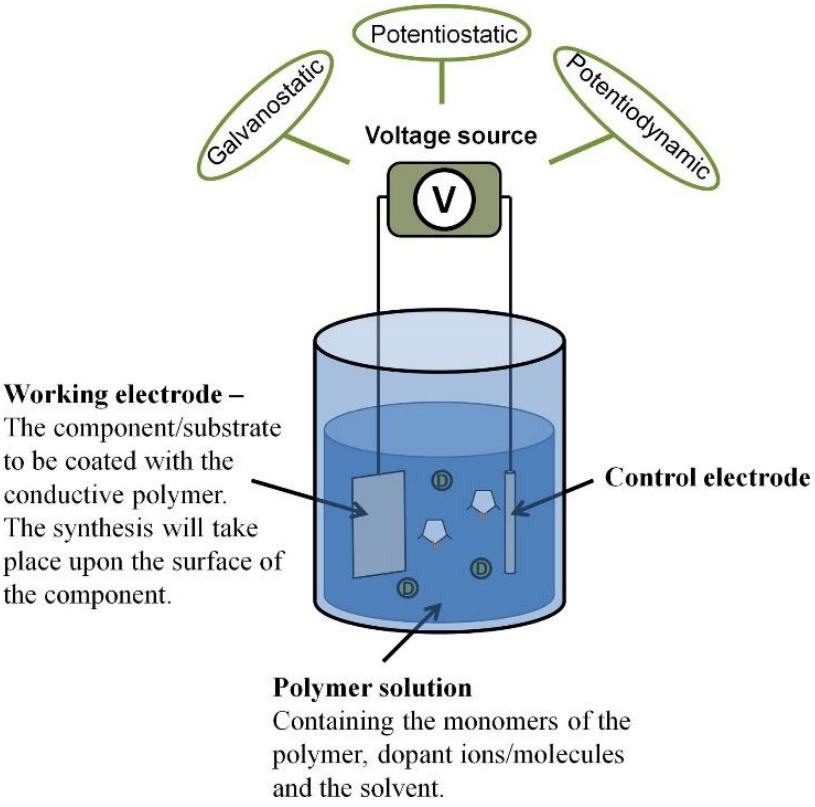


Fig. 5: The electrochemical synthesis of CPs: set-up schema.
(Balint, Cassidy and Cartmell, 2014)

1.1.1.2 Polyaniline

This CP is typically prepared by the chemical oxidation of aniline with ammonium peroxydisulfate in an acidic aqueous medium (Stejskal and Gilbert, 2002). The oxidation of aniline is an exothermic reaction, and the temperature of the reaction mixture increases during its course. This can conveniently be used to monitor the progress of polymerization (Stejskal *et al.*, 2015). The electrochemical synthesis of PANI, as well as chemical synthesis, is always carried out in strongly acidic solutions (pH < 2). Otherwise, the increase in pH would lead to the formation of short conjugation oligomers (Fedorko *et al.*, 2010; Blinova *et al.*, 2007). Morphology, conductivity and redox properties of obtained PANI are usually affected by anions. These anions are inserted during electropolymerization of aniline and they originate from present acid (Mandić, Duić and Kovačiček, 1997; Spinks *et al.*, 2002).

Both aniline oligomers and PANI are insoluble in the aqueous reaction medium and they separate (in a form of powder) during the synthesis. However, the precipitation is not the disordered agglomeration of insoluble polymer (Stejskal *et al.*, 2015).

In addition to conductivity, PANI is a very interesting polymer due to various morphology emerging in dependence on polymerization conditions (Fig. 6). It can, for example, form globules (Kadam and Patil, 2018), nanofibres (Hui *et al.*, 2017), or nanotubes (NTs) (Sim and Choi, 2015).

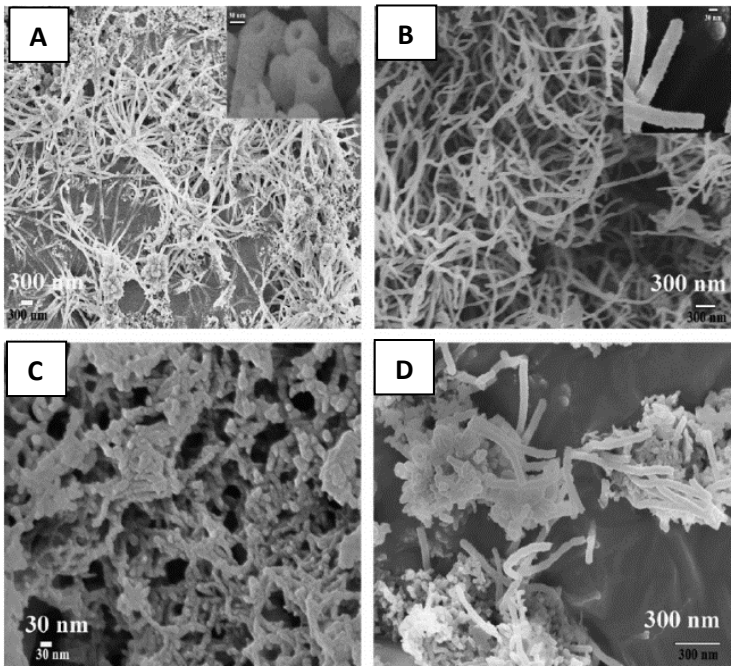


Fig. 6: The scanning electron microscopy (SEM) micrographs of different morphologies of PANI (A) NT (higher length) (B) nanofiber (C) interconnected network structure (D) NT (lower length). (Garai and Nandi, 2009)

By removing or adding electrons through chemical or electrochemical oxidation and reduction it is possible to obtain forms of PANI with different chemical structures, stability, coloring, and electrical properties. PANI can exist in several forms based on its oxidation and protonation level as following: the fully oxidized pernigraniline base, half-oxidized emeraldine base, the 75% intrinsically oxidized nigraniline and fully reduced leukoemeraldine base (Fig. 7) (Ghasemi-Mobarakeh *et al.*, 2011). It is also well known that PANI in the emeraldine oxidation state can be reversibly switched between its electrically non-conducting (base) and conducting (salt) forms. Due to the combination all of these aspects of this CP with many other advantages, such as easy and economic preparation, and nanoscale morphologies of PANI, makes this polymer an object of ever-increasing number of research papers (Stejskal *et al.*, 2015; Lai *et al.*, 2016; Gong *et al.*, 2018; Li *et al.*, 2018).

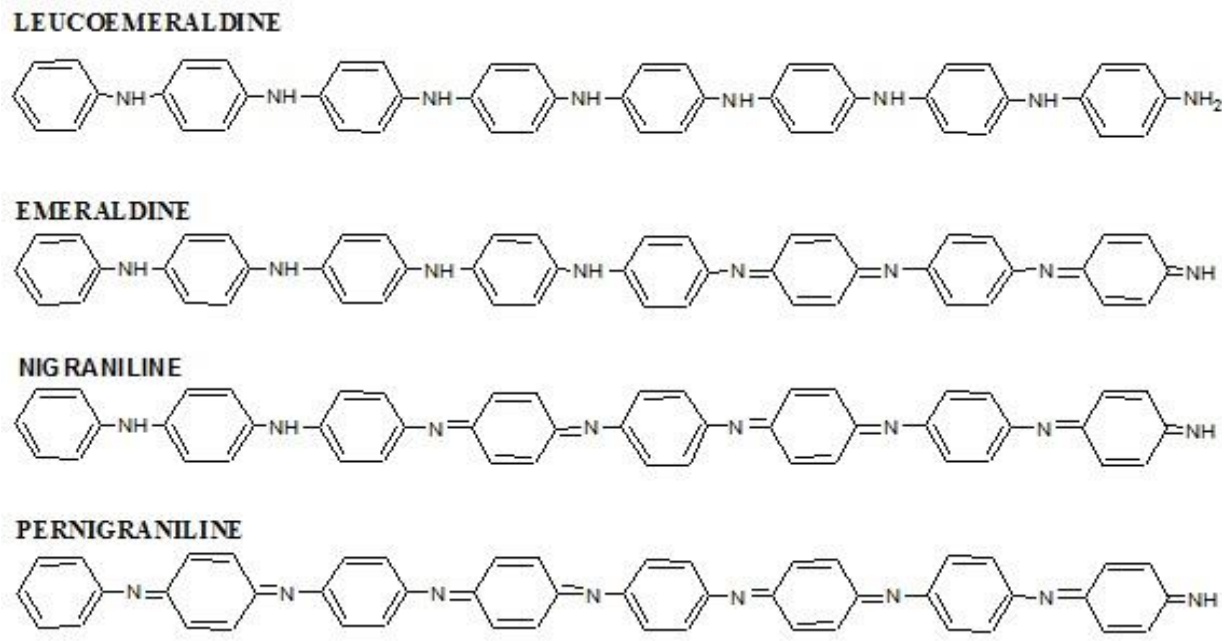


Fig. 7: The structures of PANI in various intrinsic redox states.
(modified according to (Kang, Neoh and Tan, 1998)

In addition to polymer powder, PANI can produce a thin film, which is also an interesting application form. Any surface which is immersed in the reaction mixture used for the PANI preparation becomes coated with a thin film of this polymer (Fig. 8).

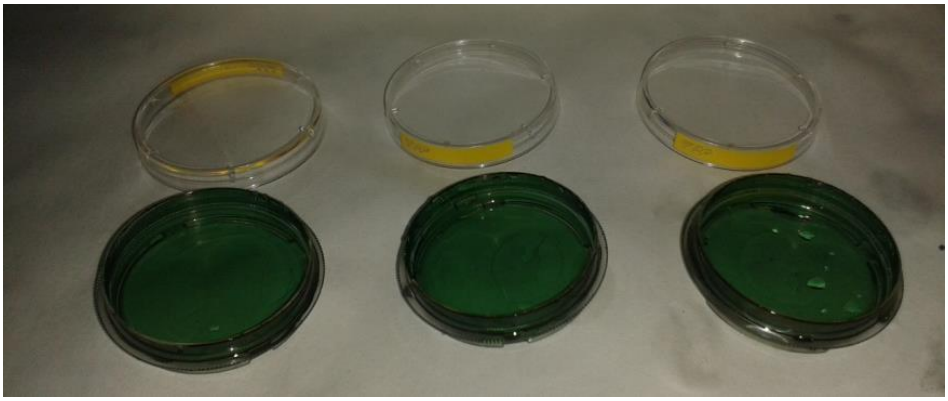


Fig. 8: Conducting PANI films prepared at the laboratory of the Centre of Polymer Systems by Nikola Mikušová.

The film formation begins with adsorption of nucleates onto support and the film is subsequently produced. In Fig. 9, the triangles represent the oligomeric nucleates. These nucleates are generated in the aqueous medium and adsorb on available solid supports. Then, PANI chains grow from them to produce a PANI film (Tomšík *et al.*, 2012). Thin films are assumed to have a brush-like structure (Sapurina, Riede and Stejskal, 2001); film thickness is of 100–400 nm depending on reaction conditions (Stejskal *et al.*, 1999). The PANI films are

more uniform when formed on hydrophobic surfaces. On the other hand, the polymeric film formed on hydrophilic substrates tends to have a globular structure (Stejskal *et al.*, 2015). More information about PANI films is given in chapter 1.1.2.1.

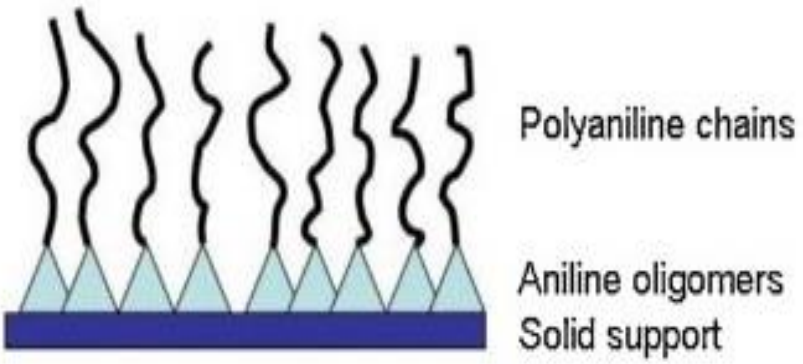


Fig. 9: Formation of PANI films. (Tomšík *et al.*, 2012)

The most common forms of this polymer are conducting, green-colored, emeraldine PANI salt (PANI-S) and nonconductive blue emeraldine base (PANI-B). The transformation of PANI-S to the PANI-B takes place under alkaline conditions and the conductivity decreases from units of S cm^{-1} to 10^{-9} S cm^{-1}. The advantage of the salt-base transition is its reversibility. PANI-B can be reprotonated with any sufficiently strong acid to obtain PANI-S again. The process of reprotonation is simple. PANI-B is immersed in the aqueous solution of acid with sufficiently high acidity, pH < 3, to form the salt. For that reason, salts are produced with most common inorganic acids and strong organic acids, such as sulfonic, phosphoric or nitric acids (Stejskal, Prokeš and Trchová, 2008; Stejskal, Prokeš and Trchová, 2014). The carboxylic acids, however, do not usually produce analogous salts with PANI. Both PANI-S and PANI-B differ not only in electrical properties but also in their behavior in contact with living organisms, tissues or individual cells (Kumar, Singh and Yadav, 2015; Balint, Cassidy and Cartmell, 2014; Jaymand, 2013).

Due to the ability to respond to external stimuli, by changing the conductivity, PANI belongs to stimuli-responsive polymers (Dong, Han and Choi, 2018). Therefore, its application is directed towards the field of sensors, anticorrosion coatings or catalysis of organic reactions. Applications of PANI include fields of supercapacitors (Zhou *et al.*, 2005), microwave materials (Oyharcabal *et al.*, 2013), electronic devices (Kumar *et al.*, 2016), sensors (Li *et al.*, 2004) or composite membranes (Blinova *et al.*, 2012).

Another promising use of this polymer is in biomedicine, especially for development and research in the regeneration of the heart or nervous tissue (Stejskal *et al.*). Other applications of PANI can be seen in Fig. 10.

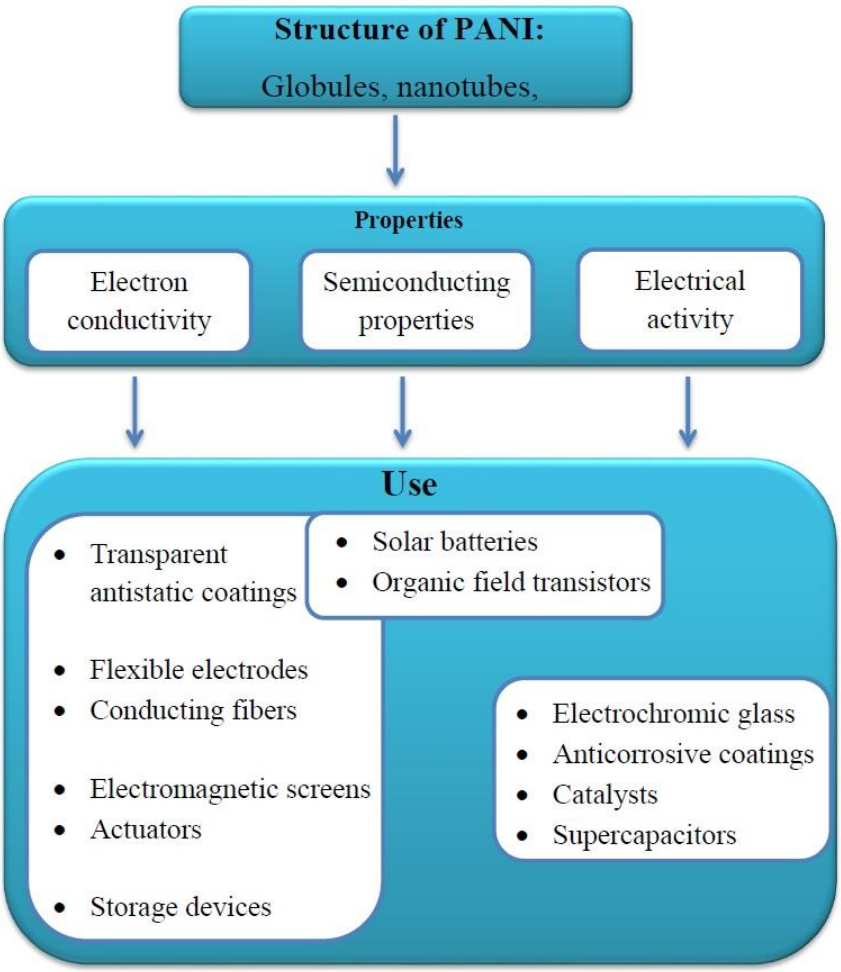


Fig. 10: Application of PANI.
(modified according to (Boeva and Sergeyev, 2014)

Recent reports have also shown that PANI possesses antimicrobial effects. The antimicrobial activity of aniline-based CPs was explained through the electrostatic adherence between polymer molecules and the microorganisms, as they carry charges of different signs. By the interaction of polymer and microbial cells, the walls of the microorganism break down and the intracellular fluid leaks out, causing their death (Gottesman *et al.*, 2011; Nanlin Shi, 2006).

PANI is also important polymer thanks to its potential anti-corrosive activity. In the early 80s of the last century, the first work on the corrosion protection of metals by CPs was reported by Mengoli *et al.* (Mengoli *et al.*, 1981) and DeBerry (DeBerry, 1985) who studied the behavior of PANI electrodeposited on steel. These scientists found out that there may be a considerable potential behind using CPs as anti-corrosion coatings. Nowadays, protective coatings having anti-biofouling and anti-corrosive features are considered as really effective methods for solving problems with corrosion of metals using in a water environment (Banerjee, Pangule and Kane, 2011). Thanks to their significant bactericidal and antifouling properties, heavy metals such as zinc, tin or copper

are the most commonly used biocides for antifouling coatings (Karlsson, Ytreberg and Eklund, 2010; Antizar-Ladislao, 2008). Therefore, a combination of the above-mentioned metals with non-conventional antimicrobial agents, such as PANI, gives the possibility to prepare attractive, effective and inhibitory functional coatings (Montemor, 2014).

1.1.1.3 Poly(phenylenediamines)

Poly(phenylenediamine) (PPDA) with the formula given in Fig. 11 is an electroactive polymer of the aromatic diamines family. Phenylenediamines show excellent diversity caused by the main part of these materials – diamines. PPDA can exist in three isomer forms – ortho, meta and para; differing in chemical, physical, mechanical and thermal properties (Stejskal, 2015).

PPDA can be prepared by oxidation of respective monomers to oligomers and polymers (do Nascimento, Sestrem and Temperini, 2010).

Several properties of PPDA are similar to PANI, owing to the similarity of the structures (e.g. redox activity), although, PPDA is substantially less conducting compared to PANI (Stejskal, 2015; Kohl and Kalendová, 2015). The low level of conductivity is an important reason why the usefulness of PPDAs has not still be proved in various applications, such as corrosion protection in comparison with other CPs (Stejskal, 2015). Consequently, their copolymers were tested more frequently (Yao *et al.*, 2019; Domínguez-Aragón *et al.*, 2018).

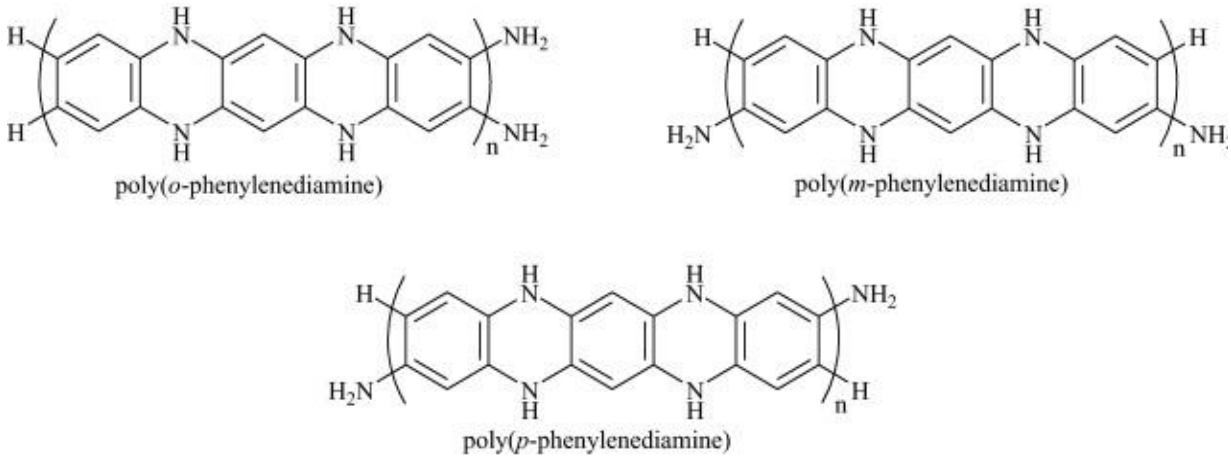


Fig. 11: Chemical structures of poly-(meta-phenylenediamine), poly-(ortho-phenylenediamine) and poly-(para-phenylenediamine).
(Duran, Bereket and Duran, 2012)

The other interesting features of this polymer are the ability to adsorb heavy metal ions and high environmental stability (Amer *et al.*, 2015). Correspondingly to other CPs, its applications are also found in electronic devices such as supercapacitors and batteries (Jaidev and Ramaprabhu, 2012). PPDA has also demonstrated potential for use as a smart material with

electrochromic properties, and in protection against metal corrosion (Rehim *et al.*, 2011).

Nowadays, there is not enough information on PPDA. Reported publications on the antimicrobial and biological activity of this polymer are also sparse. However, PPDA has a potential, similarly to PANI and PPy, to become promising antimicrobial agents and to be successfully used in biomedical applications (Al-Hussaini and Eldars, 2014; Kucekova *et al.*, 2017).

1.1.1.4 Polypyrrole

Another CP is polypyrrole (PPy) (Fig. 12) whose synthesis by chemical oxidation was described already in 1887. This reaction provided, however, only oligomer products (Dennstedt and Zimmermann, 1887; Prokeš, Stejskal and Omastová, 2001). The next significant period in CP development occurred firstly after 1979 when Diaz *et al.* published an electrochemical way of PPy synthesis (Diaz, Kanazawa and Gardini, 1979; Kanazawa *et al.*, 1980).

The oxidation of pyrrole leading to preparing PPy is governed by the same principles as oxidation of PANI, and their formal chemistry is similar (Blinova *et al.*, 2007). As it was already stated above, PPy can be also prepared using electrochemical synthesis (Vernitskaya and Efimov, 1997; Parakhonskiy and Shchukin, 2015). Unlike polymerization of aniline, polymerization of pyrrole can be successfully performed in a neutral aqueous environment even with using various organic solvents (Vernitskaya and Efimov, 1997). Correspondingly to PANI, also PPy can create thin films with a thickness lower than 1 μm. These films own also several spectral properties depending on the conditions of synthesis and degree of PPy oxidation. With the increasing oxidation degree, the color of the films changes from yellow to blue and, ultimately, black (Diaz, Kanazawa and Gardini, 1979). The electropolymerized films have good adhesion to a substrate. Although, at a thickness of over 10 μm they are relatively easily peeled off the electrode (Vernitskaya and Efimov, 1997). The film adhesion depends on a number of factors including the nature, coarseness, hydrophobicity of the electrode surface and the solvent used (Scharifker, García-Pastoriza and Marino, 1991). The properties of PPy, such as electrical conductivity and chemical stability, originate from heteroatomic and extended π-conjugated backbone structure. Unfortunately, the structure of PPy alone is not sufficient for conductivity. Therefore, doping of this polymer allows for increasing conductivity that exceeds those of other CPs (Gvozdenovic *et al.*, 2014; Khanh *et al.*, 2018).

PPy is frequently used due to its good conductivity, excellent environmental stability, *in vivo* and *in vitro* biocompatibility, stability under *in vivo* conditions, redox behavior and reversible protonation. Correspondingly to PANI, PPy exhibits the unique combination of electrical properties, flexible method of preparation, easy surface modification and ion exchange capacity (Omastova *et al.*, 2014). The environmental stability of PPy is associated with the reactivity of

the charged polymer backbone towards oxygen or water. Owing to the low oxidation potential of PPy, the sensitivity of redox reaction to oxygen is higher in the case of PPys compare to those polymers that are more difficult to oxidize (Ansari, 2006; Skotheim, 1986).

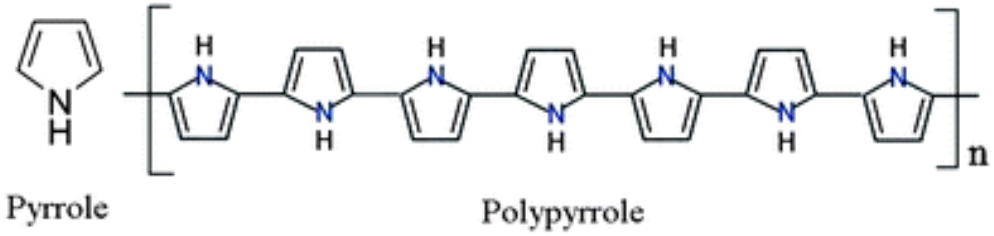


Fig. 12: Structures of pyrrole monomer and PPy.
(Miah, Iqbal and Lai, 2012)

Besides PANI, PPy is certainly one of the most extensively investigated CP (Li *et al.*, 2016; Rikhari, Pugal Mani and Rajendran, 2018; Istakova *et al.*, 2019). As regards applications, PPy is suitable for tissue engineering, such as the fabrication of implants with satisfactory strength or flexibility (Ungureanu *et al.*, 2014). PPy is also applicable in chemical sensors, biosensors or gas sensors, ion-selective electrodes, batteries and conducting coatings for nanomaterials (Armelin *et al.*, 2008). In addition, it can be often used in wires (Mahmoodian *et al.*, 2019), electronic devices (Huang *et al.*, 2016) or functional membranes (Arroyo *et al.*, 2019). PPy, as well as PANI and other CPs, provides a possible answer to the demand for "green" corrosion inhibitors for decreasing the health risk to humans and damage to the environment (Pan, 2013). Compared to PANI, PPy has reasonably high conductivity in a wide pH range. Due to this fact, PPy provides outstanding corrosion protection abilities (Chamovska, Porjazoska-Kujundziski and Grchev, 2013; Nautiyal *et al.*, 2018; Garcia-Cabezon *et al.*, 2020).

PPy has also been considered as a potential candidate for applications involving biofilm detection and control (Cordeiro *et al.*, 2015). In the research of Khan *et al.* (Khan *et al.*, 2019), chitosan-PPy composites were synthesized to inhibit and influence the biofilm formation of *P. aeruginosa*. The aim of the study was also to clarify the mechanism of antimicrobial activity of virgin PPy. It was found that proved inhibition of biofilm formation may be caused by the electrostatic interactions between positively charged composites and negatively charged groups present on the bacterial membrane. Though there are few studies reported on this topic, the presence of positive charge on the backbone of PPy is a reason why this polymer can act as an antimicrobial agent (Varesano *et al.*, 2013; Varesano *et al.*, 2015).

1.1.2 Anticorrosive properties of polymeric materials

1.1.2.1 Polymeric films

As was already mentioned, the CPs show very significant feature, namely the ability to easily coat various surfaces. This process consists in immersion these surfaces in a polymer reaction mixture. Subsequently, the formation of CP films begin. These thin films are still developed and the technique of their preparation for subsequent applications is still improving. The physical properties of the films differ substantially from the intrinsic behavior of the corresponding polymers in bulk (Hashim, 2010). Furthermore, polymeric film technology has a key role in helping to understand the cell-surface interactions. It then leads to the elucidation of how living cells respond to the surfaces.

Despite the popularity of CPs, certain properties of these polymeric materials and practical problems still limit their use such as hydrophobicity, poor solubility and processability, uncontrollable mechanical properties and inability to degrade (Guimard, Gomez and Schmidt, 2007; Thomas *et al.*, 2000; Green *et al.*, 2012; Kishi *et al.*, 2012). For this reason, the polymeric films can be modified through several techniques (see chapter 1.1.3).

The antibacterial and antifungal activities of polymeric films are equally important. Hence, there is needed to develop material that does not allow microorganisms to attach and proliferate on its surface (Muthusankar *et al.*, 2018; Zengin *et al.*, 2019).

1.1.2.2 Polymer-based coatings

In addition to the formation of polymeric films, CPs can be used for the preparation of polymeric coatings containing also additives (fillers, pigments, stabilizers; see chapter 4.2). Polymeric coatings can be functional (adhesives), protective (anticorrosion) or decorative (paint) (Kalendová *et al.*, 2015; Francis and Roberts, 2016).

Even if metals are frequently used materials, their corrosion is a huge threat in daily life and leads to financial losses. From the thermodynamic point of view, corrosion is an energetically favorable process that converts a high-energy metal into its low-energy oxide form. Thanks to this fact, it is practically impossible to prevent corrosion (Rohwerder, 2009).

Nowadays, coatings containing anti-corrosion agents, such as pigments, are used for corrosion protection (Prokeš and Kalendová, 2007). The effectiveness of the anticorrosive pigments depends on the pretreatment of a metal surface, the type, the concentration of the pigments, the film formation process, the adhesion of the coating onto the metal base and the mechanical properties of the entire coating system (Veselý, Kalendová and Němec, 2010).

But during the time coating defects can occur at the interface between coating and metal. In order to limit the corrosive attack, the organic coating applied on metallic surfaces consists of additives inhibiting corrosion (Rohwerder, 2009).

The corrosion can be also influenced by the microbial contamination of the metal surface. It is, therefore, important to study also the coatings which can prevent the aggressive microbial species from attacking a metal surface (Advincula, 2015). These coatings should have very good barrier properties, be free from any defects and preclude to aggressive microorganisms, ions, water, and corrosion products to diffuse through and be strongly adhered to the metal surface (Advincula, 2015). Figure 13 shows the appearance of the selected coatings and the metal substrate after the corrosion test.

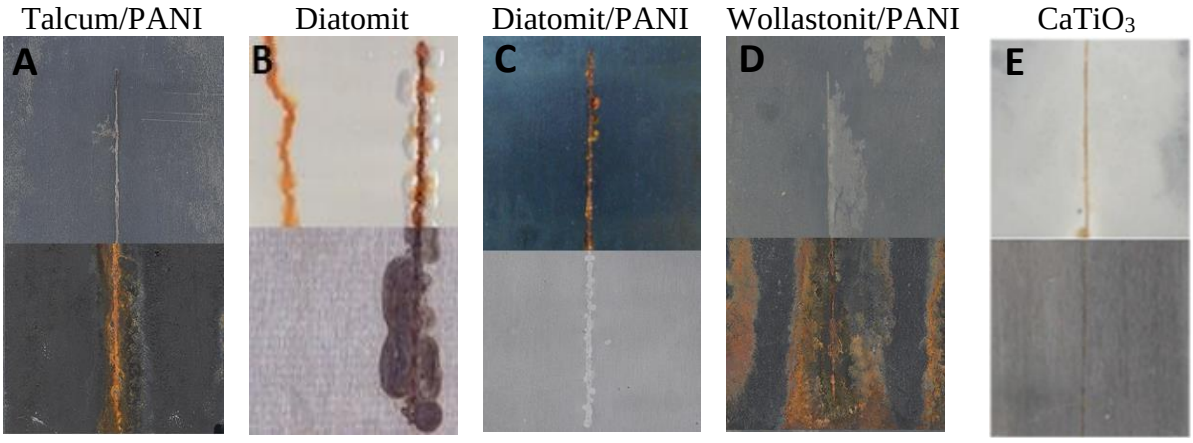


Fig. 13: The appearance of the selected coatings and the metal substrate after the corrosion test. The coatings are shown in the upper half of the panel, steel panel is shown on the lower half. (Department of Paints and Organic Coatings, University of Pardubice)

Nowadays, the effort is directed towards complying with ecological requirements and replacing the paints containing traditional anti-corrosive pigments based on Pb, Cr^{+VI} (e.g. zinc tetraoxychromate, zinc yellow) with ecologically compatible pigments. Therefore, chemically and toxicologically acceptable substitutes are being sought. One possibility is application of CPs or treatment of suitable pigments with CPs. Unlike other macromolecular substances, CPs have barrier properties and porous structure. Thus, they are highly permeable to liquids and gases (Kalendová *et al.*, 2014). PANI furthermore belongs to the CPs that are the most investigated for anticorrosive activity (Sokolova *et al.*, 2017; Deshpande and Sazou, 2016). For example, corrosion-resistant hydrophobic coating based on modified PANI was investigated in the work of Adhikari *et al.* (Adhikari *et al.*, 2017). They polymerized aniline in the presence of dopant phenylphosphonic acid (PPA) by two methods: conventional and rapid mixing methods. Subsequently, PANI-PPA was mixed with epoxy resin to make a homogenous dispersion of coating. This coating was then applied to mild steel. As a result, the polarization curves showed that the polymeric coating induced a passive-like behavior and

increased the corrosion resistance of the PANI-PPA/epoxy-coated mild steel in the aggressive NaCl solution. It was revealed that the anticorrosive protection of the epoxy coating was enhanced by adding CPs.

The good anticorrosive properties of PANI on carbon steel were also confirmed by Shi *et al.* (Shi, Zhang and Yu, 2017) who prepared hydrophobic PANI coatings modified with SiO_2. The superior anticorrosive effect was ascribed to the electrochemical activity of PANI. In the study of Grari *et al.* (Grari *et al.*, 2015) the multilayered PPy-SiO_2 composite coatings for the functionalization of stainless steel were synthesized and the anticorrosive protection of PPy with incorporated SiO_2 was also verified. The main target of the research team of Chen (Chen *et al.*, 2017) was to investigate the anticorrosion performance of epoxy coating which contained reactive poly(o-phenylenediamine) nanoparticles. These particles proved the improvement of anticorrosive resistance of tested coatings on Q235 steel. Kalendová *et al.* (Kalendová *et al.*, 2015) prepared zinc-filled epoxy coatings containing CPs (PANI, PPy) and pigments. The results of this study confirmed that treatment with CPs had a beneficial effect on the mechanical properties of the coating material.

1.1.3 Modification of conducting polymers

CPs offer many advantageous properties over the other materials, as it was already mentioned within this doctoral thesis. However, the additional modification and optimization of CPs are also required. Optimizing of properties of CPs is important with respect to their targeting to specific applications. The most significant properties of CPs with respect to biomedical fields cover mainly the conductivity, biocompatibility and redox stability (Guimard, Gomez and Schmidt, 2007). Surface modification techniques were also developed to impart antimicrobial properties on polymeric films and coatings. One of the modifications relies on reprotonation with various dopant acids (Stejskal, Prokes and Trchova, 2008), thereby altering their biological properties (Humpolicek *et al.*, 2015; Bober *et al.*, 2015). Modification, overall, can change the physical, chemical, mechanical and material properties, functionality, nanostructure of CPs, etc. Since the polymeric surfaces are often non-reactive, and surface modification may involve chemical alteration of the surface layer, it requires the generation of high-energy species. These high-energy species include radicals, ions, and molecules in an excited electronic state to promote a surface reaction. This is enabled by using modification techniques such as flame, plasma, UV, laser, X-ray, electron beam or ion beam (Sharma, Sims and Mazumder, 2002). Modification using sterilization (e.g. moist and dry heat, UV or ethylene oxide) is another possibility of how to change the characteristics of CPs.

1.1.3.1 *Chemical modification*

PANI, which is the most investigated CPs within this doctoral research, can be easily modified by the chemical way (Fig. 14).

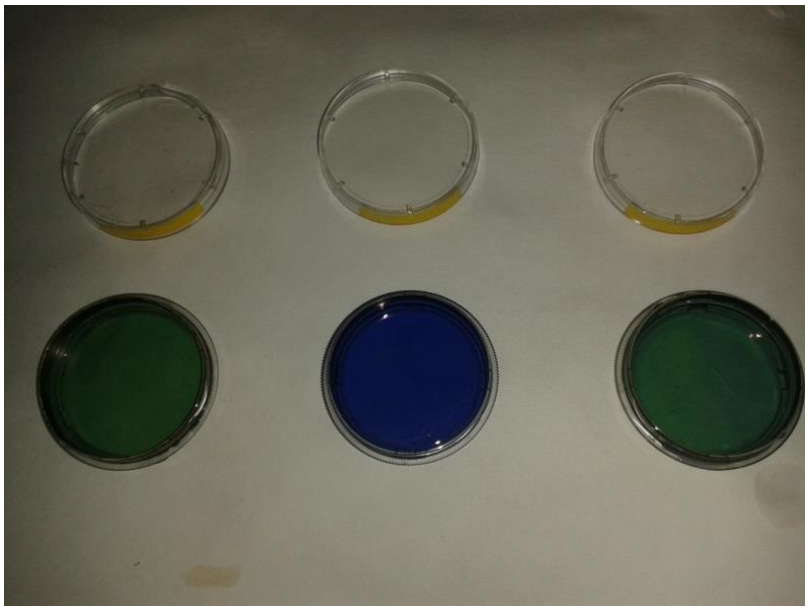


Fig. 14: PANI films prepared at the laboratory of the Centre of Polymer Systems by Nikola Mikušová. From left to right – green PANI-S, blue PANI-B and green PANI-S doped by PAMPSA.

One of the PANI forms, PANI-B, is soluble in some polar organic solvents. However, its conductivity is very low. On the other side, the PANI-S shows reasonable conductivity but it is practically insoluble and is considered as unprocessable. PANI-B may be doped by acid-base chemistry with various acids. It can, for example, include protonic organic acids containing long alkyl side chains, such as camphorsulfonic acid (CSA), dodecylbenzene sulfonic acid (DBSA), PAMPSA or phosphotungstic acid (Jaymand, 2013; Pyo and Hwang, 2009; Ayad and Zaki, 2008; Humpolicek *et al.*, 2015). The reprotonation using mentioned doping acids (Fig. 15) has been working well and due to these long alkyl chain acids, resulting PANI can be both conducting and soluble in some nonpolar or weakly polar organic solvents (Jaymand, 2013). Simply said, these long chains behave as plasticizers. Further, these chains have other advantageous features, as they provide PANI with bulky space and improve the miscibility of PANI-based blends (Jaymand, 2013).

Depending on the type of acid, the reprotonation of PANI can offer materials, that vary in conductivity, hydrophobicity, density and other physicochemical parameters (Stejskal, Prokeš and Trchová, 2014; Stejskal, Prokes and Trchova, 2008).

The research team of Cabuk *et al.* (Cabuk and Gündüz, 2017) prepared and tested chemically synthesized PANI doped by boric acid for controlling the optical properties. Kashyap *et al.* (Kashyap *et al.*, 2019) synthesized ascorbic

acid-doped oligoaniline and its drug composites for studying their antibacterial behavior. The oligoanilines were prepared using the chemical oxidation method. Antibacterial properties of tested materials were investigated against four gram-positive bacteria with importance in clinical and pharmacological practice (*S. aureus* MTCC 96, *S. pyogenes* MTCC 442, *B. subtilis* MTCC 441 and *S. mutans* MTCC 890) and four gram-negative (*E. coli* MTCC 443, *P. aeruginosa* MTCC 1688, *Kl. pneumoniae* MTCC 109 and *S. typhi* MTCC 98).

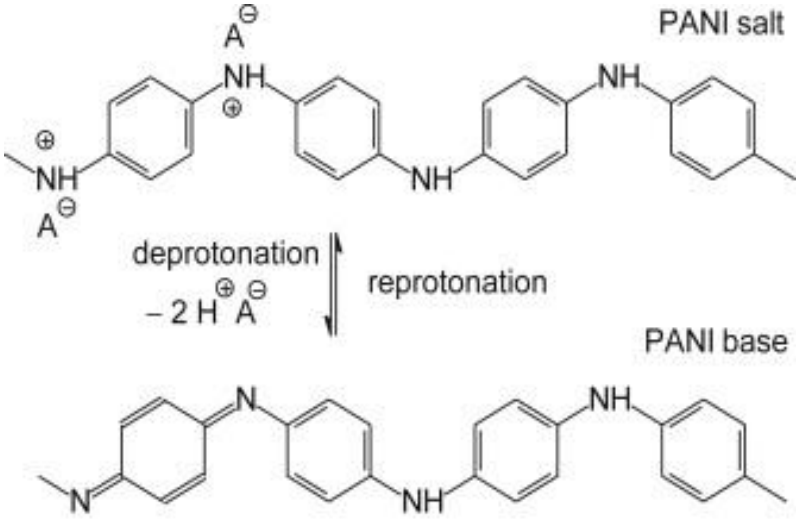


Fig. 15: Conducting PANI-S deprotonation in alkaline media to PANI-B. The base can be again reprotonated with acids (HA) to corresponding salts. (Stejskal, Prokeš and Trchová, 2014)

Introducing sulfonic acid groups in PANI chains can be considered as another successful approach toward the preparation of soluble conducting PANI (Jaymand, 2013). It has been established in several studies where PANI has figured as the target of considerable recent interest, mainly due to better processability (Atkinson *et al.*, 2000) and good electrical properties (Wen, Huang and Gopalan, 2001). On the other hand, the unwanted reaction after this sulfonation process is decreasing the electrical conductivity of PANI. The negative resonance effect of the sulfonic acid groups (the sulfonic acid groups withdraw electrons from the benzene ring) is the probable reason for decreased conductivity (Jaymand, 2013).

As regards PPy, its coatings electrochemically synthesized on carbon steel using sulfonic acids as dopants were prepared by Nautiyal *et al.* Dopants as p-toluene sulfonic acid, sulfuric acid, CSA, sodium dodecyl sulfate (SDS) and sodium dodecylbenzene sulfonate were tested. The main aim was to investigate corrosion protection and possible application of resulting materials as biocides. The dopants significantly affected the protection efficiency of the coating against chloride ion attack on the metal surface. It was found out that p-toluene sulfonic acid and sodium dodecylbenzene sulfonate were the most appropriate dopants for PPy coating on carbon steel. It was probably due to the presence of

aromatic rings from the dopants ions. Moreover, the PPy coating demonstrated the potential biocidal function. It could be applicable for reducing of microbiologically influenced corrosion. PPy coating doped with sodium dodecylbenzene sulfonate showed excellent biocidal abilities against *S. aureus* (Nautiyal et al., 2018). Further, in the study of Bhadra *et al.*, the high-performance sulfonic acid doped PANI-polystyrene blend ammonia gas sensors were evaluated (Bhadra *et al.*, 2016).

1.1.3.2 *Plasma treatment*

Polymers, in general, show various benefits, such as low density, flexibility, ease of manufacture, and cost-effectiveness. Nevertheless, in the case of CPs, it is necessary to meet the demands regarding their scratch-resistance, wettability, biocompatibility, gas transmission, adhesion, or fiction (Hegemann, Brunner and Oehr, 2003). Therefore, in addition to chemical modification, plasma treatment using various gases (N_2, O_2, Ar, He) is also an important method for surface modification of CPs. Primarily, the plasma treatment provides manifold possibilities to refine a polymer surface. This is achieved by adjustment of plasma parameters, such as gas flow, power, pressure and treatment time (Hegemann, Brunner and Oehr, 2003). During plasma treatment, the action of energetic particles occurs. The important advantage of this process is that during treatment only the top of the surface (few nm) is modified. Moreover, the modification unifies the surface characteristics, whilst the bulk properties are left unchanged (Junkar *et al.*, 2009). Plasma influences the surface properties, such as chemical composition, morphology, and wettability. All of them lead to improvements in the biological response of materials. This fact plays an essential role in the process of adhesion both prokaryotic and eukaryotic cells (see Fig. 16). Plasma treatment can further provide polar functional groups on the polymer surface. It subsequently alters the surface energy of polymers.

Plasma modification has also a disadvantage, namely the aging effect of the treated material (Junkar *et al.*, 2009). This aging effect is influenced by several external factors (such as adsorption or oxidation) and on the internal tendency to attain an energetically favorable state (Hegemann, Brunner and Oehr, 2003). The research team of Zaitsev evaluated PANI films treated by argon plasma to obtain nanostructured surfaces. Their results confirmed that the density and the dimensions of the nanostructures can be tuned by the plasma operating parameters (Zaitsev *et al.*, 2017).

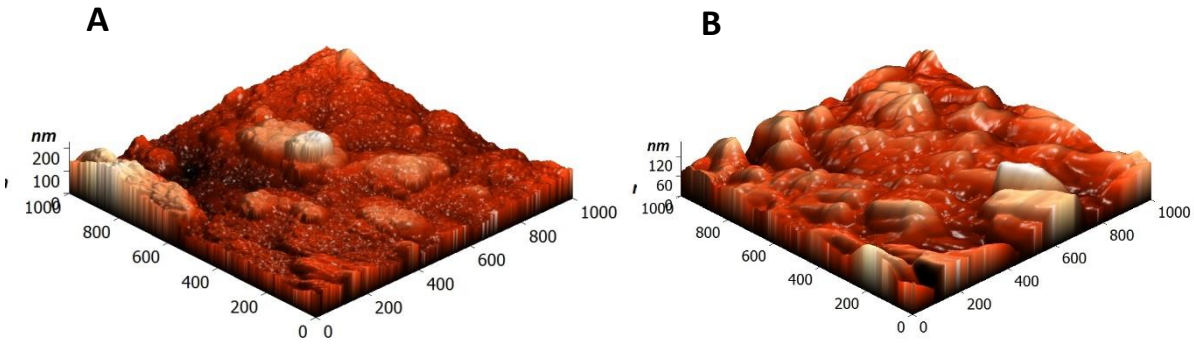


Fig. 16: Atomic force microscopy (AFM) micrographs (1x1 μm^2) of PANI-S films – untreated (A) and oxygen plasma treated for 30 sec. (B) showing surface roughness reduction. AFM evaluation was performed in cooperation with the Laboratory of Surface Engineering and Optoelectronics, Jožef Stefan Institute in Ljubljana, Slovenia.

1.2 Biological properties of polymeric materials

In coatings and polymeric films, besides other factors, there is an emphasis on the interaction of the material surface with microorganisms. The colonization of the surface by microorganisms can have an unfavorable impact on its functionality. Microbial adhesion can cause corrosion, degradation or failure of the material.

Microbial adhesion can further lead to biofilm formation if the microorganism species is biofilm-forming. Moreover, biofilm may initiate the formation of biofouling. Hence, the best way to control biofouling is to prevent the formation of biofilms before it starts.

Microorganisms (bacteria, fungi) clump together with an intention to build certain protection of their colony, also especially on wetted surfaces. This microbial attachment called biofilm is further described in more detail in chapter 1.2.1. This unwanted microbial adhesion is, therefore, the main reason why a lot of area in industry, biomedicine and many others, endeavor to develop antibiofilm materials. The second mentioned problem, the biofouling[1], causes serious problems worldwide, as the deterioration of materials and the affecting of human health (Bachmann and Edyvean, 2006).

This doctoral study focused on polymeric films and coatings with the aim to influence their antibiofilm properties (see 6: Experimental part). Moreover, the surfaces of films and coatings can be subsequently modified (see chapter 1.1.3.) to improve properties particularly related to the antibiofilm activity.

1.2.1 Biofilm

Similarly, as people build the cities, microorganisms build their own world – biofilm (see Fig. 17). In this structure, microorganisms have many beneficial features and take advantage of these benefits. Unfortunately, some properties of biofilm are not merely beneficial for human life. The disadvantages of biofilm are as following: causing damages in industry, attacking the human body and organism, parasitizing activities of the human body and in some cases causing fatal infections. Finding possibilities for biofilm prevention and treatment is therefore the subject of many research studies (Flemming *et al.*, 2016; Satpathy *et al.*, 2016; Watnick and Kolter, 2000).

[1] Biofouling is fouling of any deposit (e.g. microorganisms, macroorganisms, plants, animals) or the undesirable accumulation of biotic matter onto a surface in contact with liquid, including biofilm formation (Flemming, 2002; Fusetani, N., 2004).

1.2.2 Definition and structure of biofilm

Microorganisms, especially bacteria, generally exist in two types of population: planktonic or biofilm-forming. In planktonic form, bacteria move individually, thus it can seem that microbial cells could abound in many advantages due to free movement. However, bacteria in biofilm, compared to planktonic bacterial cells, have gained other benefits such as the higher resistance to the environment factors. In substance, biofilm formation is a mode of growth by which the microorganisms protect themselves and survive in hostile environments (Hall-Stoodley, Costerton and Stoodley, 2004). The biofilm formation and its development are an important survival strategy for every biofilm-forming microbial cell lasting over millions of years. This process is designed to anchor microorganisms in a nutritionally advantageous environment. It permits their escape if the essential growth nutrients have been exhausted. Biofilm further allows cells to survive in hostile environments and colonize new niches (Wei and Ma, 2013; Toyofuku *et al.*, 2016).

Although the descriptions of biofilm have varied over the years, the fundamental characteristics are as followed:

- complex and dynamic structure,
- the communication between individual microorganisms in biofilm using biochemical signaling molecules,
- less susceptibility to antimicrobial agents,
- different gene expression compared with planktonic microorganisms.

Biofilm can be therefore defined as a community of sessile colonies of microorganisms on solid or semi-solid substrates of natural and synthetic origin surrounded by polysaccharide layer called extracellular polymeric substance (EPS) (Fig. 17) (Hall-Stoodley, Costerton and Stoodley, 2004).

The biofilm structure is composed of several components. Primarily, bacterial cells are indeed the most important element in biofilm. Further, to ensure proper biofilm structure, the structure of biofilm should contain EPS accounting for 50-90 % of the total organic carbon. This matrix material varies in chemical and physical properties. Polysaccharides are the main part of EPS (Rodney, 2002). The structures of biofilms differ and every microbial community is unique (Tolker-Nielsen and Molin, 2000). Nevertheless, some structural characters can generally be considered universal (Rodney, 2002). Biofilm-forming microcolonies consist of individual species populations or multimember communities of microbial species depending on the environmental parameters (Costerton, 1999). Surface and interface properties, nutrient availability, the composition of the microbial community or hydrodynamics and many other conditions may affect biofilm structure (Stoodley *et al.*, 1997).

The composition and quantity of the EPS depend on the type of microorganisms, biofilm age and the environmental conditions in which the microorganisms exist (Mayer *et al.*, 1999). The different components of EPS also influence the extent to which microorganisms may adhere and create biofilm onto hydrophobic and hydrophilic surfaces (Rodney, 2002). In addition, most microorganisms have been found to attach more rapidly to hydrophobic and nonpolar surfaces (Teflon) compared with hydrophilic surfaces (glass or metals) (Flemming and Wingender, 2001).

The interstitial voids and channels are another integral part of biofilm simply providing the lifeline of the whole biofilm system. Voids and channels help to circulate nutrients and exchange metabolic products with the bulk fluid layer inside the biofilm structure (Costerton, 1995). The internal biofilm structure is given by a heterogeneity. The biological material is situated in clusters containing cells and the excreted polymeric network. Whereas channels and pores filled with the ambient liquid besiege free spaces between the clusters (Mara and Horan, 2003). Each of the clusters may contain layers with different microbial species, polymer compositions or densities of active cells (Keevil and Walker, 1992).

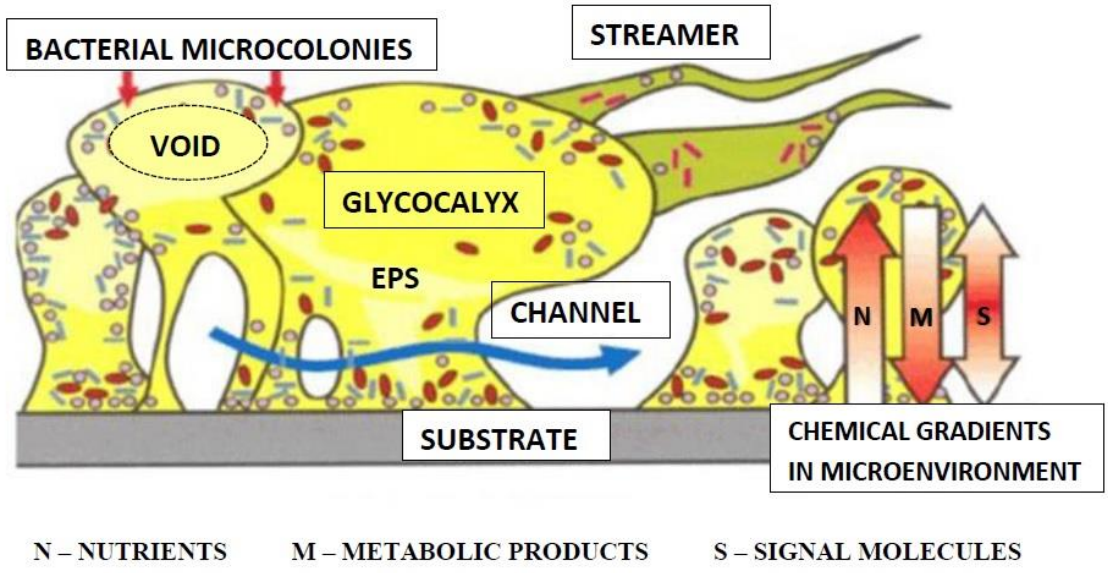


Fig. 17: Schematic representation of the structure of a mature biofilm. (modified according to (Kanaparthy and Kanaparthy, 2012)

1.2.3 Biofilm formation

The adhesion of microorganisms can be divided into two distinct phases – primary and secondary. The primary or docking stage is reversible and is determined by a number of physiochemical variables. Whereas the secondary stage or locking stage adhesion becomes irreversible if there is no physical or chemical interference. By the end of these two phases, the main biofilm formation occurs (Dunne, 2002). Figure 18 describes the sequence of reactions

and interactions showing the progress in the attachment of bacterial cells onto the substrate.

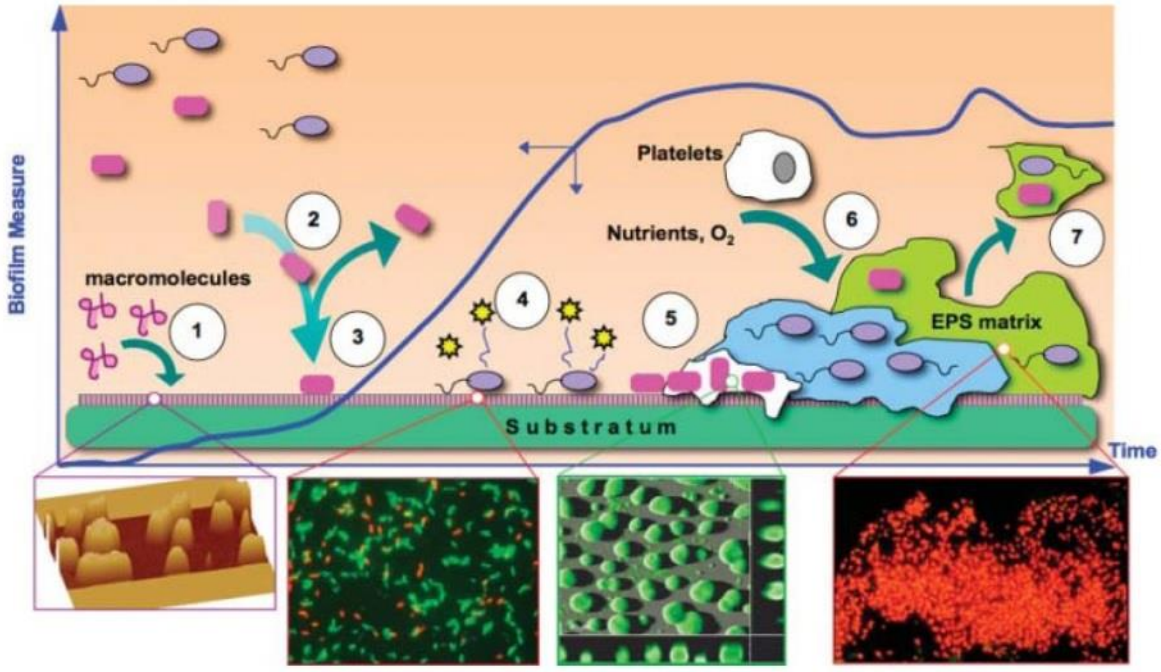


Fig. 18: Phases of biofilm formation. (Bryers, 2008)

Step 1: Firstly, for the occupying of the surface, it is essential to recognize the distance between bacteria and material where they want to adhere. Therefore, they usually use signaling molecules that can not penetrate into a solid surface. Subsequently, their concentration increases in the vicinity. In addition, surface properties play also a key role. Bacteria prefer to settle a rough surface due to elevations and smaller shearing forces. Moreover, it is most preferred to adhere to nonpolar hydrophobic surfaces, whose settlement is simpler and faster for bacterial cells (Costerton, 1999; Rodney, 2002).

Step 2: In another step, bacteria realize the adhesion to the selected surface by reversible forces (as van der Waals forces, steric interactions, and electrostatic interaction). They examine if the selected material fulfills the conditions necessary for survival (Costerton *et al.*, 2003).

Step 3: If these unicellular organisms are decided that the surface provides all conditions for subsequent attachment, they begin to settle permanently by non-specific and/or specific receptor: ligand adhesion mechanisms. On the contrary, if the material does not provide the survival conditions, the cells are released back to the environment. So they have still an effort to find another suitable object to settle down (Bryers, 2008).

Step 4: Within a few minutes, bacterial cells, already adhered to the surface, start to up-regulate the secretion of certain cell signal molecules. This complex

process is called quorum sensing. The signaling molecules are released and other bacteria, as well as from other species, are attracted (Costerton, 1999).

Step 5: As the next step of biofilm formation, the up-regulation of virulence factors and secretion of extracellular polymers occur. After a significant change in the microbial cell genotype, the bacterial cells produce large amounts of EPS (Costerton, 1999).

Step 6: Biofilm formation continues. Sessile bacteria to the surface utilize the soluble nutrients from the environment. Moreover, bacteria try to recruit other bacterial species or mammalian cells to join the biofilm (Bryers, 2008).

Step 7: Biofilm formation is completed. However, the fragments of biofilm and microcolonies may be detached depending on other quorum cell signals. Subsequently, these fragments are carried downstream (Bryers, 2008).

The biofilm formation and its composition are also influenced by the interplay between the species of microorganisms, their growth, type of substrate and fluid on a surface. The composition of the surrounding environment is another aspect affecting the interactions during the attachment of microorganisms onto a surface (Fig. 19) (Renner and Weibel, 2011).

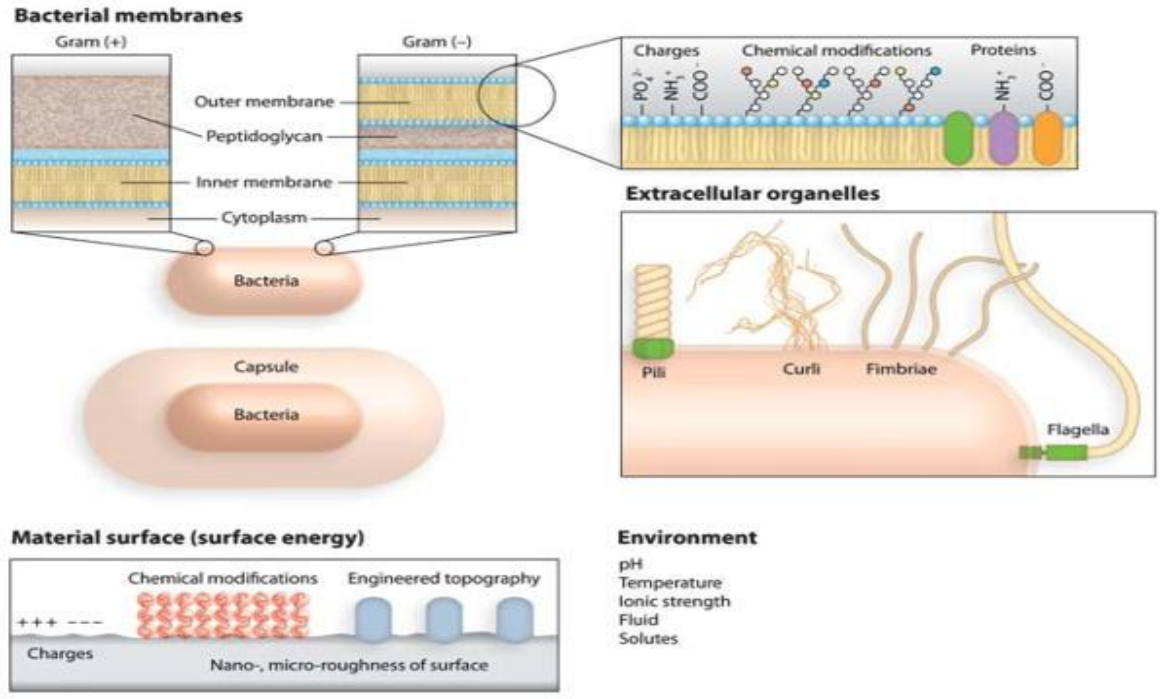


Fig. 19: Parameters influencing the interactions between bacteria and surfaces. (Renner and Weibel, 2011)

Many important factors also facilitate or impede the adhesion of microbial cells on a substrate. There are physical, chemical and biological interactions including specific and non-specific reactions. The cell growth is affected by the conditions as temperature, fluid flow, carbon source and composition of nutrient media and growth factors. However, these factors may vary with microbial species and extracellular conditions (Guo, Liu and Su, 2011; Bruellhoff *et al.*, 2010).

The effort to manipulate individual environmental factors to prevent undesirable biofilm formation has increased in recent years (Toyofuku *et al.*, 2016). Even, the control over surface chemistry was used to reduce cell attachment (Renner and Weibel, 2011). Although these strategies are in great development, it is hardly possible to irreversibly eliminate the attachment of microorganisms and prevent the biofilm formation (Bridier *et al.*, 2011).

The microorganisms are diverse. In addition, their prevalence is terrific. They are able to adapt to their extracellular conditions. This fact enables them to establish themselves in almost all habitats in the biosphere, including humans. The microorganisms were forced to develop such mechanisms to attach the surfaces and form biofilms due to survival in diverse and fluctuating conditions (Hall-Stoodley, Costerton and Stoodley, 2004). Bacteria in a biofilm can be protected by polysaccharides from a wide range of stresses, and predators such as phagocytic cells (Flemming and Wingender, 2010). That is the main reason why biofilm becomes persistent and difficult to remove. In recent decades, scientists and people in the industry have efforts to understand mechanisms of microbial growth.

1.2.4 Quorum sensing

Quorum sensing is a significant factor in biofilm formation as well as other properties. The quorum sensing system allows cells to synthesize and transmit signaling molecules called autoinducers (Fig. 20). These molecules provide information to other cells. Upon reaching a specific population density, the level of concentration of the signal molecules increases. It gives the impulse to change the behavior of the entire population. The quorum sensing intensity depends on cell density and also on the external influences (Li *et al.*, 2004; Li and Tian, 2012). Moreover, gram-positive and gram-negative bacteria have different types of communication systems. In the case of gram-positive strains, bacteria use secreted oligopeptides and two-component systems. These two-component systems are composed of membrane-bound sensor kinase receptors and cytoplasmic transcription factors. Consequently, alterations in gene expression occur. Whereas, in the case of gram-negative bacteria, several autoinducers are used in the quorum sensing system. Small RNAs are also integrated into quorum sensing information. These small RNAs control target gene expression (Rutherford and Bassler, 2012).

One possible way how to stop biofilm formation is to influence the quorum sensing. Respectively to block the signaling molecule synthesis or to interfere with the signaling molecule metabolic pathway. This inhibition can be done by:

- affecting of signaling molecule production,
- binding of the signaling molecule to its receptor,
- conformational change of the receptor protein,
- binding of receptor protein and DNA (Chelikani, 2007).

This antibiofilm method is still not fully explored. However, it opens up a new technique to prevent microbial biofilm formation (Chelikani, 2007).

1.2.5 Difference between bacterial and fungal biofilm formation

As well as the bacterial biofilm formation, the fungi in their biofilm community have become an increasingly significant economic, industrial and clinical problem (fungal biofilm is shown in Fig. 20). Fungi belong biologically and evolutionarily to different taxonomic domains - bacteria are Prokaryota and fungi are Eukaryota.

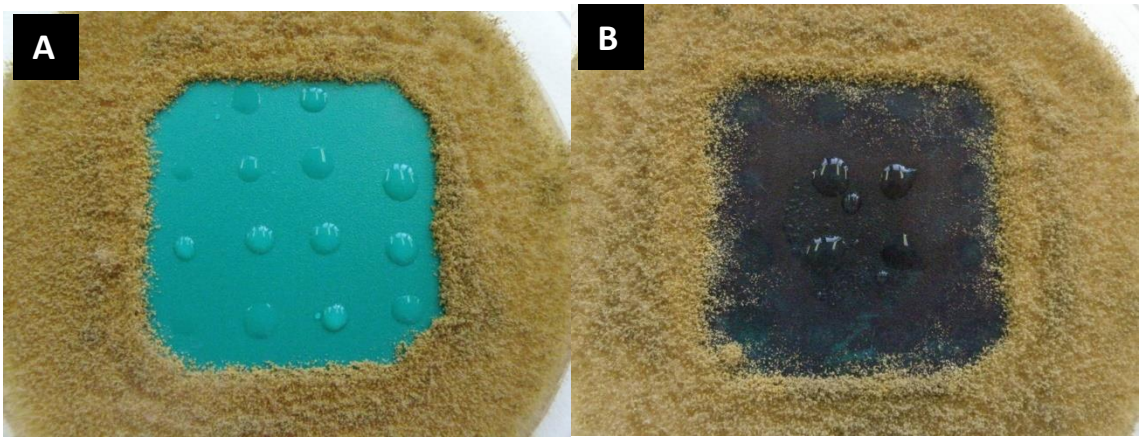


Fig. 20: Fungal biofilm formation: *Aspergillus niger* on polypropylene (A) and *Aspergillus oryzae* on PANI (B). (The images were taken in the laboratory of Microbiology, the Centre of Polymer Systems, Tomas Bata University (TBU) in Zlín)

Fungal strains contain chitin that is unique for fungal cells. It plays a key role in the stabilization process of the glucans network around the fungal cell membrane – it protects the cell membrane. Hereby, chitin provides structural integrity and it is involved in hypha production and sporogenesis (Coad *et al.*, 2014).

The filamentous fungi produce not just the extracellular enzymes that are released because of the degradation of substrata where the fungal strains are attached, but also a large number of molecules such as mycotoxins. Mycotoxins are very harmful to other organisms and they have toxic effects (Ravikumar *et al.*, 2006).

The researches focusing on the fight against biofilm formation of filamentous fungi have been done previously only to a limited extent - the filamentous fungi are not intensively studied compare to bacterial strains (Lugauskas, 2003; Binkauskiene, Lugauskas and Bukauskas, 2013). The impact of fungal strains and possible prevention against their negative effect is discussed in more detail in the practical part of this thesis.

1.2.6 The resistance of microbial biofilm

Significant influence is also attributed to the physical and chemical constraints of the environment. Among the most important factors belongs:

1) Physical surface properties - regulate cell attachment and physiology and affecting the early stages of biofilm formation.
2) Chemical properties - influence the cell adhesion to surfaces and their development into biofilms.
3) Chemical communication between cells - attenuates growth and influences the organization of communities and biofilm formation (Renner and Weibel, 2011).

Nowadays, microorganisms in the biofilm structure are becoming more and more resistant to antimicrobial agents or antibiotics/reactive molecules. These agents/molecules are produced by the host immune systems (Rabin *et al.*, 2015). The production of the exopolysaccharide matrix is one of the crucial factors influencing the resistance of microbial biofilm. This is caused because of the fact that this matrix prevents the access of antibiotics to the microbial cells embedded in the community. These multiple mechanisms vary with the presence of microbial strains in the biofilm, and the drug or biocide, which is applied. The multiple mechanism includes physical or chemical diffusion barriers to antimicrobial penetration to the biofilm (Mah and O'Toole, 2001). Mechanisms of biofilms resistence to antibiotics and biocides are divided into four classes (Fig. 22) (Jamal *et al.*, 2015):

a) active molecule inactivation directly,

b) altering the body's sensitivity to the target of the action,

c) reduction of the drug concentration before reaching the target site,

d) efflux systems.

Figure 21, further, shows that antibiotic resistance is associated with biofilm and describes the significant keys of mechanisms. These key mechanisms are involved in antibiotic resistance. As a sample, it can be mentioned following: limited diffusion, enzyme causing neutralizations, heterogeneous functions, slow growth rate, presence of persistent (non-diving) cells and biofilm phenotype such as adaptive mechanisms (Jamal *et al.*, 2015).

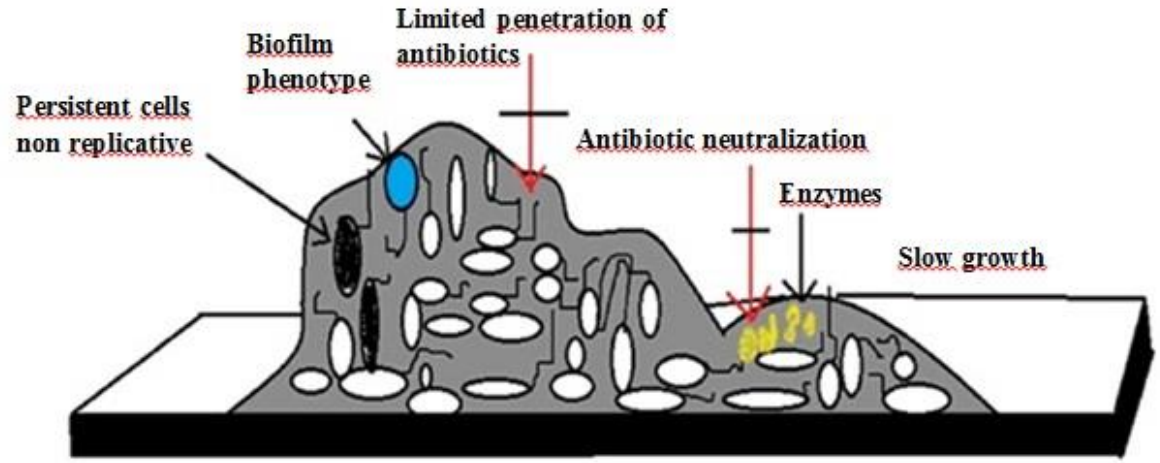


Fig. 21: Antibiotic resistance associated with biofilms.
(Jamal *et al.*, 2015)

1.3 Surface properties affecting biofilm formation

The attachment of microbial cells onto any surface is the first and critical step of biofilm formation. Thus, the surface properties of materials play a very important role from the beginning (Fig. 22) (Kochkodan and Hilal, 2015). Microbial cells get to the surfaces using several ways such as Brownian motion, sedimentation, movement with the liquid flow, microbial motility with cell surface appendages, interaction with other cells to form aggregates (Teughels et al., 2006).

From a material perspective, the surface roughness, topography, surface free energy, surface charge, electrostatic interactions, and surface hydrophobicity are generally known to be relevant parameters for the attachment process (Rummel et al., 2017). To develop some suitable materials, that are applied in biomedical fields or in industry, it is also needed to understand the structure and chemistry of the solid-liquid interface (Pavithra and Doble, 2008). These factors are related to biofilm formation and biofouling mainly because of the determination of the interaction between the surface and the foulants (Kochkodan and Hilal, 2015).

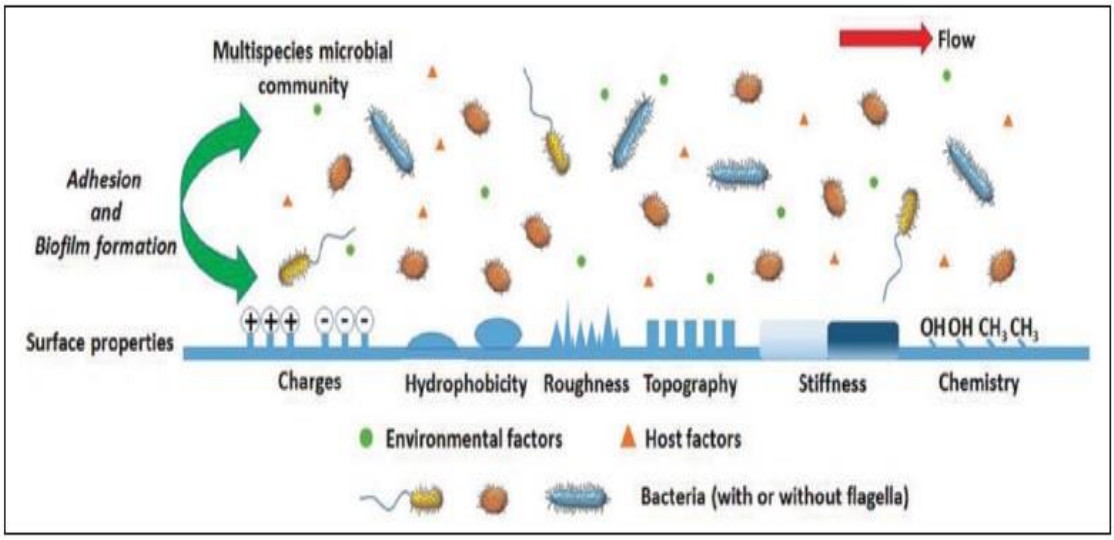


Fig. 22: Bacterial adhesion/biofilm formation and the effects of material properties in complex environments. (Song, Koo and Ren, 2015)

1.3.1 Surface charge

Surface energy plays an important role in determining the binding force between microorganisms (in case of this thesis – bacteria), and the surface. It is known that most bacterial cells have a negative charge. The negative charge is on their cell wall, though the charge magnitude differs from strain to strain. Thus, a more preferred material for their adhesion has to be positively charged. It follows from this finding that negatively charged surfaces are more resistant to bacterial attachment (Campoccia, Montanaro and Arciola, 2013). These surfaces repeal bacterial adhesion due to electrostatic repulsion between the negatively charged bacterial surface and the negatively charged surface of polymer (Montag *et al.*, 2012). It means that the adhesion of bacterial cells will take place only under the condition when the resulting electrostatic repulsion is overcome by attractive forces. These forces are van der Waals forces or hydrophobic interactions between bacterial surface polymers and a solid surface (Marshall, 1986). Nevertheless, positively charged surfaces may also be bactericidal, supposedly thanks to their positive charge. So the positive charge can disrupt cell membrane potential or damage the membrane structure (Strahl and Hamoen, 2010).

Nowadays, the research papers deal with the examination of the behavior of bacteria with the negatively charged cell wall (Borthakur *et al.*, 2018; Liu *et al.*, 2018). It is because of their more extensive occurrence in nature and the environment. Although, in the past, the attachment of the positively charged wall bacteria to a material surface was also examined (Jucker, Harms and Zehnder, 1996).

Surface energy itself does not directly influence the binding of cells on a surface, but it influences the binding of proteins (e.g. onto biomaterial) which

subsequently affects the binding of cells. Adsorption of proteins onto surfaces is one of the crucial factors in the adhesion process not just in the case of eukaryotic cells, but also in case of prokaryotic cells. Protein adsorption, similar to biofilm formation, is influenced by surface energy, hydrophobicity, intermolecular forces and other interaction important for cell adhesion. Therefore, if we know how the protein adsorption is affected by these factors, they can be modified by different engineering techniques. Subsequently, the optimal surface properties are obtained for biomedical applications.

A human body is composed of fluids containing different proteins. Constantly, these proteins have mutual competition for adsorption to any exposed biomaterial surface. Despite the fact that the adsorption is a very complex process, it possesses many advantages. One of the important benefits of protein adsorption is that it can enrich a biomaterial surface with the proteins possessing the highest affinity to it. The competitive adsorption behavior of proteins is important to many interfacial phenomena such as biocompatibility (Felgueiras *et al.*, 2018). Typically, protein-adsorption takes place in a highly dynamic process with constantly adsorbing and desorbing proteins. As already published in 1962, the "Vroman-effect" postulated that the identities of adsorbed proteins are able to change over time but the total amount of adsorbed proteins is more or less stable (Vroman, 1962). Vroman effect is simply the most common phenomenon involving competitive protein exchanges on surfaces (see Fig. 23). Molecules contained in a protein mixture diffuse to a surface at different rates. However, the proteins arriving as a first one may be then displaced from the surface by proteins that subsequently come to adsorb on the same substrate. Due to higher affinity to the surface, big size and flexibility in conformation, these proteins capable of displacing early adsorbed proteins are able to adhere strongly to the material (development of more contact points). The initial protein population is so replaced by new proteins at the end of this adsorption process. Therefore, the final composition of the newly adsorbed layer is a result of the type, amount and relative affinity of proteins recruited from and available in the solution, as well as their reversible adsorption character (Felgueiras *et al.*, 2018).

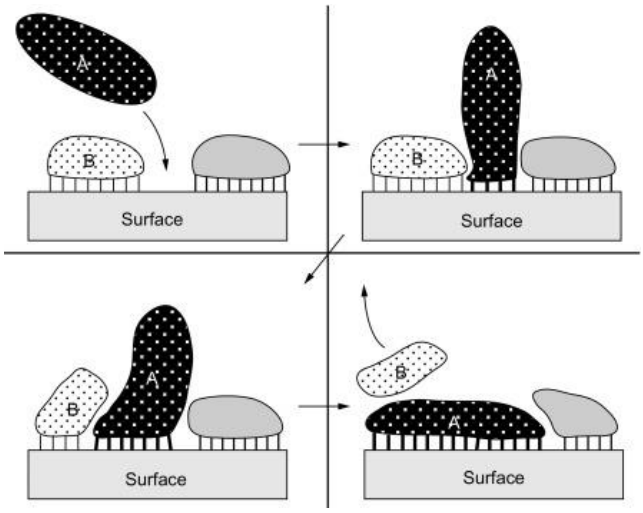


Fig. 23: The Vroman effect - protein B arriving first to the surface, subsequently it is displaced by protein A creating more stable bonds with the surface available binding sites. (Felgueiras *et al.*, 2018)

1.3.2 Roughness and topography

The increasing surface roughness promotes the cells to attach onto a surface. This is predominantly due to the increase in the contact area between material and bacteria (Anselme, Ploux and Ponche, 2010); and protection from shear forces (Teughels *et al.*, 2006). Therefore, the reduction of bacterial biofilm formation can be achieved by smoothening of the surface. It may contribute to significant prevention against undesirable bacterial attacks (Song, Koo and Ren, 2015).

The main effects of material roughness on biofilm adhesion and formation differ in the environmental factors. Thanks to this fact, the universally optimum roughness, that could suppress the adhesion of all bacterial species, does not exist (Renner and Weibel, 2011). The effect of the surface chemical composition and topography on the adhesion and viability of *Pseudomonas aeruginosa* was studied by Gallarato *et al.* (Gallarato *et al.*, 2017). The bacterial viability was decreased on the PANI surface in comparison with the substrate (polyethylene terephthalate). In addition, bacterial adhesion was decreased even more upon the micro-structuring of PANI films. Thus, the biofilm reduction could be improved using polymers with different chemical composition and/or the same polymer with different topographies. The research paper of the team of da Silva deals with the influence of different preparation procedures of PPy. It was on the action of resulting antibacterial composite against bacterial strains such as *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*. The morphology of PPy, especially the size of its nanoparticles, was reported as the main reason for bactericidal effects. The electrostatic interaction established

between polymer nanoparticles and bacteria provokes the bacterial cell death. These found results showed PPy as a strong potential candidate for fast bactericidal actuation against bacteria (both gram-positive and gram-negative) compared to a conventional complex of metal/polymer nanocomposites (da Silva Jr *et al.*, 2016).

1.3.3 Biofilm prevention

Many scientists deal with microbial biofilm and effort to find new and effective procedures. Especially, they want to find out how to decrease the number of undesirable microbial biofilms onto substrates. In the following subchapters, surface modification and surface structuring will be discussed in more detail.

1.3.3.1 Surface modification

The modification of material may achieve the increasing of material biocompatibility and on the other hand the decrease of biofilm formation. For more details see chapter 1.1.3. In Fig. 24, there are shown various surface modification techniques.

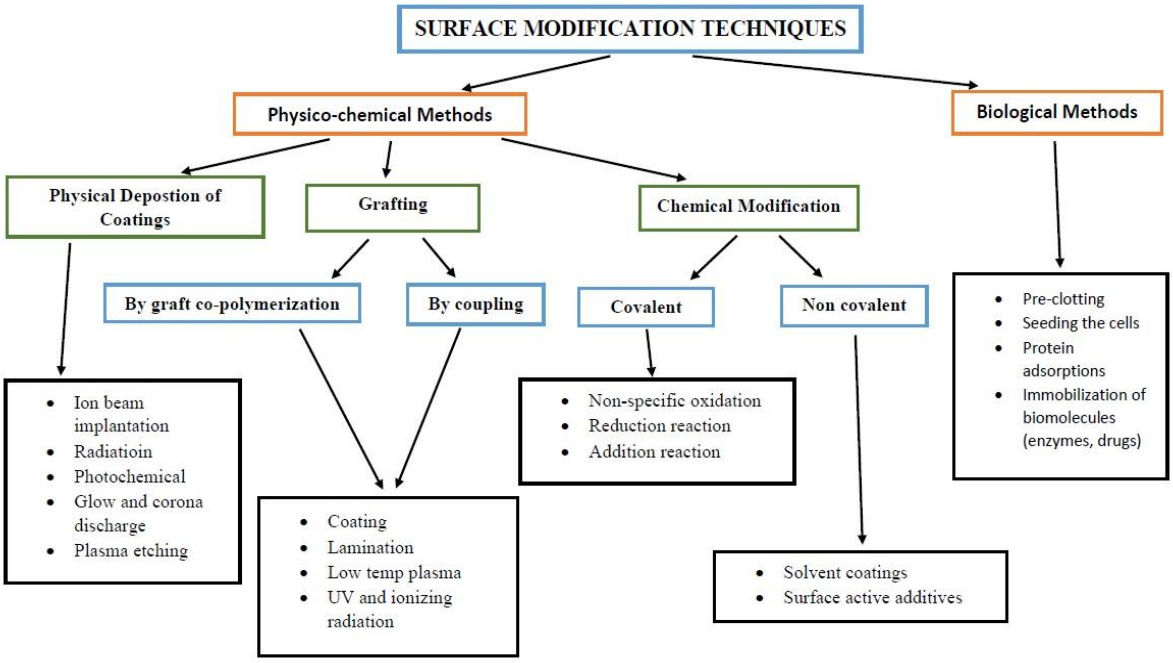


Fig. 24: Various surface modification techniques.
(modified according to (Pavithra and Doble, 2008)

1.3.3.2 Surface structuring in relationship with antimicrobial properties

An unexhausted source of inspiration for research is found in nature - biomimetics. Biomimetics is the field of scientific endeavor, that attempts to design systems and synthesize materials through biomimicry (Rao, 2003). Furthermore, in biomimetics, hybrid technologies are developed by using the tools of molecular biology and nanotechnology. Traditionally, this field is inspired by biological structures and their functions. It is focused mainly on emulating or duplicating biosystems using mostly synthetic components and following traditional approaches (Sarikaya *et al.*, 2003; Wanieck *et al.*, 2017). Nature provides a vast array of biological materials and materials systems. These materials systems have inspired innovations in novel applications and in new materials developments. It includes natural armor, flight systems inspired of course by birds, fasteners and attachments. Further, the biomimetics takes inspiration in plant leaves, gecko foot, shark skin, insect wings, fish scale, and spider silk (Liu and Jiang, 2011; Fullenkamp *et al.*, 2012; Jaggessar *et al.*, 2017; Hu *et al.*, 2017; Murr, 2015). Therefore, natural surfaces having low adhesive, superhydrophobic and self-cleaning properties are in the center of interest due to their potentially anti-biofouling characteristics (Hasan, Crawford and Ivanova, 2013).

As a sample of natural animal surfaces, cicada wings were chosen due to their considerable surface properties (see Fig. 25). Their surface seems to be bactericidal to bacteria *Pseudomonas aeruginosa*. The surface nanostructure plays the most important role in the bactericidal activity of this insect (Ivanova *et al.*, 2012; Jindai *et al.*, 2019). Although the bacteria are able to attach onto the wing surface, they are consequently mechanically ruptured during a short time. That happens because of the action of the particular surface nanopattern. Therefore, the nanostructure of cicada wings in interaction with bacterial mechanism, which has been recently proposed, could have big potential in the production of antibacterial surfaces (Pogodin *et al.*, 2013).

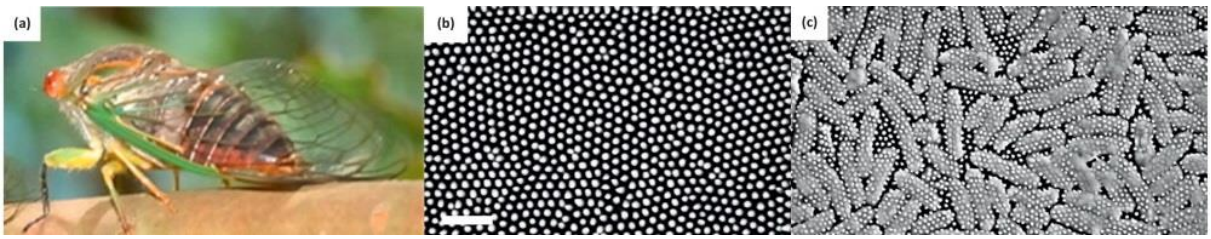


Fig. 25: The surface of cicada wings. (a) Photograph of a cicada. (b) Scanning micrograph of the hexagonal arrangement of nanopillars on the cicada wing surfaces, scale bar = 200 nm. (c) Interaction of the *Pseudomonas aeruginosa* cells with the wing surface, scale bar = 1 μm.
(Hasan, Crawford and Ivanova, 2013; Pogodin *et al.*, 2013; Ivanova *et al.*, 2012)

The antibacterial or antibiofilm surface can be also modified using antibacterial agents (nanoparticles, polymeric compounds) such as silver, titanium dioxide (TiO_2) or metal oxides (Mg, Zn), to improve antimicrobial activity. There is a claim why these nanoparticles (NP) behave as bactericidal. That is due to their electrostatic forces, basic character, oxidizing power of halogens, generation of reactive oxygen species, and accumulation of nanoparticles near the cytoplasm, that kills the bacterial cells (Parashar *et al.*, 2011; Modaresifar *et al.*, 2019; Netala *et al.*, 2015; Manna *et al.*, 2015). For example, nanoparticles of TiO_2 are not active in the dark condition. The bactericidal activity is stimulated by photoactivation. Visai et al. (Visai *et al.*, 2011) prepared a review of titanium oxide antibacterial surfaces in biomedical devices. They offer a comparative analysis of the use of TiO_2 as a coating for materials in biomedical devices. Whereas, a review of Chouirfa (Chouirfa *et al.*, 2019) deals with titanium surface modification on techniques and coatings for antibacterial applications. In the chapter of nanostructures and surface of paper of Chouirfa, structuring is mentioned that controlled anodized Ti NT formation and heat treatment are strong candidates for the design of future implantable materials. These materials have improved tissue growth properties and antimicrobial behavior. The antibiofilm activity of TiO_2 is further described and examined within the experimental part (see chapter 4.3).

2 AIMS OF DOCTORAL THESIS

The doctoral thesis is focused on the understanding of critical factors influencing the interaction between microorganisms and material surfaces. Special attention is paid to the formation and growth of biofilm-forming bacterial and fungal strains. Followed issues were concerned:

- Preparation of polymeric surfaces using chemical polymerization.
- Modification of surface properties of tested materials (polymeric surfaces, polymeric coatings) and surface structuring of TiO_2 NTs.
- Characterization of material and surface properties of pure and modified surfaces.
- Determination of antibiofilm properties of surfaces using selected strains of biofilm-forming bacteria and filamentous fungi.

3 METHODOLOGY

Within the doctoral study, the experimental part was focused on the determination of antibiofilm activity of various material surfaces. In this chapter, the preparation and modification of polymeric films (such as PANI), polymeric coatings with various fillers and pigments (such as PPY, PPDA, PANI) and TiO_2 NTs are described. Further, the description of the methodology used for the evaluation of the biological properties of tested surfaces is explained below. The attachment and growth of various strains of biofilm-forming bacteria and filamentous fungi on the mentioned surfaces were tested using various quantification techniques (e.g. determination of ATP level). The antibiofilm effect of individual surfaces is discussed in the context of surface properties such as topography, surface energy, and conductivity.

Moreover, new methods were introduced to laboratory practice - comet assay and protein adsorption.

3.1 Preparation and modification of surfaces

3.1.1 Preparation of polyaniline films

Polystyrene (PS) tissue microtiter plates (96 wells; TPP; Switzerland) and polypropylene (PP) foil (16 cm^2) were utilized as substrates for preparing PANI films and subsequently tested for biofilm formation. Unadulterated PP foils and PS microtiter plates were concurrently applied as reference materials for fungi and bacteria, respectively. The coating of both substrates was performed *in situ*, in accordance with Stejskal & Sapurina (Stejskal and Sapurina, 2005), by oxidizing aniline hydrochloride with ammonium peroxydisulfate. Aniline hydrochloride (2.59 g; Sigma-Aldrich) was dissolved in water to make up 50 mL of the solution; ammonium peroxydisulfate (5.71 g; Sigma-Aldrich) was similarly dissolved to the same volume of solution. Both solutions were mixed at room temperature and poured over substrates. PANI was produced within 10 minutes. The thin films of PANI salt (PANI-S) were rinsed with 0.2 M hydrochloric acid, followed by methanol, and left to dry in air at room temperature. The PANI-S was further converted to PANI base (PANI-B) through deprotonation with 1 M ammonium hydroxide (Sigma-Aldrich, USA). Prior to the deprotonation, the samples were placed for 60 min in water in order to increase the pH of the surface, which is originally highly acidic. This treatment prevents possible mechanical deterioration of the film. It is potentially caused by the substantial difference in pH between the acidic film surface and alkaline ammonium hydroxide solution, which are associated with volume changes.

PANI films were prepared in the laboratories of the Centre of Polymer Systems at TBU in Zlín.

3.1.1.1 *Chemical modification*

PANI films were modified using the re-protonation process. For the re-protonation of the PANI-B films, three different acids were used, namely 50 wt% aqueous solution of phosphotungstic acid (PANI-PTA), and 15 wt% solution of PAMPSA (PANI-PAMPSA) (both acids purchased from Sigma-Aldrich, USA). Further, the acid solution was poured onto the surface of the PANI-B samples. After 24 h, the acid solutions were removed, and the reprotonated PANI films were washed with methanol and dried in air at room temperature. Prior to testing for biofilm formation, the films and reference surfaces were sterilized by exposure to UV-light (258 nm) for 30 min.

Modification of PANI films was carried out in the laboratories of the Centre of Polymer Systems at TBU in Zlín.

3.1.1.2 *Plasma treatment*

PANI films, whose synthesis is subscribed in chapter 3.1.1, were treated by highly reactive oxygen plasma (Fig. 26). Plasma with the oxygen gas is an effective, economically and environmentally safe modification method. This treatment leads to topography and morphology changes. Due to these impacts, the surface properties may be improved for subsequent application in medical, industrial or other fields.

Fig. 26: Oxygen plasma treatment device.
(The image was taken by Nikola Mikušová in the laboratory of Jožef Stefan Institute)

The system was evacuated with a two-stage oil rotary pump with a pumping speed of 4.4 $x10^{-3}$ $m^3.s^{-1}$. The discharge chamber was a Pyrex cylinder with a length of 0.6 m and an inner diameter of 0.036 m. The plasma was created with an inductively coupled RF generator, operating at a frequency of 27.12 MHz and output power of about 200 W. Commercially available oxygen was leaked into the discharge chamber. The pressure was measured by an absolute vacuum gauge. The pressure was adjusted during continuous pumping by a precise leak valve. In our experiments, the pressure was fixed at 75 Pa, because it was obtained the highest degree of dissociation of the molecules as measured by the catalytic probes. At these discharge parameters, plasma with an ion density of about 2 $x10^{15}$ m^{-3}, an electron temperature of 4 eV, and a neutral atoms density of about 4 $x10^{21}$ m^{-3}. The treatment was done for two times of duration (5 and 30 seconds) in the glow and afterglow regime (Fig. 27). This experiment was performed in cooperation with Jožef Stefan Institute in Ljubljana, Slovenia.

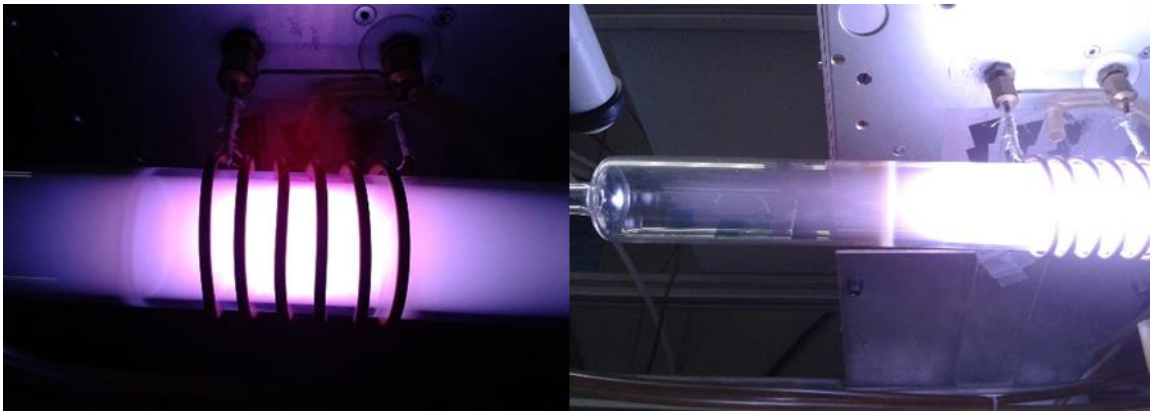


Fig. 27: Glow (left) and afterglow (right) regime of oxygen plasma treatment. (The image was taken by Nikola Mikušová in the laboratory of Jožef Stefan Institute)

3.1.2 Preparation of polymeric coatings

The commercial epoxy-ester resin Worleédur D46 (composition: 60% epoxide, 40% conjugated fatty acid, tung oil; density: 1.07 g cm^{-3}; solvent: xylene) was used as the binder for preparing the polymeric coating. Inorganic pigments coated with layers of the CP were applied as chemically active pigments. The pigments comprised the following: natural silicon dioxide diatomite (SiO_2), natural wollastonite ($CaSiO_3$), tungstate (Fe_2WO_6) and molybdate ($Fe_2(MoO_4)_3$). Tungstate and molybdate pigments were prepared by solid-phase reaction (Trojan, Brandová and Šolc, 1987) and included either iron(III) tungstate (Fe_2WO_6) or iron(III) molybdate $Fe_2(MoO_4)_3$. The pigments were modified with the following anti-corrosion agents: polyaniline phosphate (PANI), polypyrrole phosphate (PPy), poly(p-phenylenediamine) phosphate (PPDA), or $ZnFe_2O_4$ mixed oxide ($ZnFe_2O_4$). The final composition of the coatings used is summarized in Tab. 2. The composite pigments consisted of crystalline fractions (core) and X-ray amorphous fractions (shell). The pigments also contained some trace amounts of SO_3 - due to the use of the acid and/or the initiator (up to 1-2 wt.%). Further, the surface and the shape of pigments and the morphology of polymeric coatings were examined with the SEM analysis (JEOL-JSM 5600 LV, Japan).

The pigments were coated with a PANI phosphate layer, the method involving oxidative precipitation volume polymerization of aniline (Stejskal and Gilbert, 2002). For this, 9.06 ml of aniline (C_6H_7N, Fluka, Switzerland) was dissolved in 250 ml of distilled water acidified with 0.8 M H_3PO_4 (ortho-phosphoric acid, Lachema, Czech Republic). This solution was stirred and the pigment was added. A solution of 0.25M ammonium peroxodisulphate $(NH_4)_2S_2O_8$ (Lach-Ner, Czech Republic) was also prepared, and the polymerization reaction was initiated. Stirring continued for 1 hour, after which

the mixture was allowed to stand till the next day for the polymerization reaction to complete. The following day, the solids were filtered out and rinsed with 0.2 M phosphoric acid followed by acetone. The pigment particles coated with the PANI overlayer were dried in air and then at 60°C in a laboratory dryer. The composite particles contained about 10 wt.% PANI (emeraldine) phosphate. Pigments modified with layers of PPDA or PPy were prepared likewise by using p-phenylenediamine ($C_6H_8N_2$) and pyrrole (C_4H_5N) (both Sigma-Aldrich), respectively, as the initiating substances. Diatomite SiO_2 and wollastonite $CaSiO_3$ were modified with a layer of zinc ferrite, deposited on them from a ferrous salt and a zinc salt in an aqueous medium during urea hydrolysis in a basic system (Kalendová, 2002).

The model coatings were prepared by dispersing the powdered pigments in the liquid binder via a Dispermat CV pearl mill (WMA Getzmann GmbH, Verfahrenstechnik, Germany) at 3000 rpm for 45 minutes. VCP (Volume Concentration of Pigment) (ϕ_i) was 1% in all cases except the Fe_2WO_6/PANI system, where a concentration as high as 15% was applied. The polymeric (organic) coatings were obtained by applying the liquid system to a glass substrate, where they dried under the binder oxypolymerisation reaction and the physical and chemical mechanisms. Samples for measurement were prepared in thin layers by spin coating on a spin coater (Spinner, POLOS300 Advanced; Netherlands). Slides of 26 x 26 x 1 mm, previously washed and degreased with chloroform, were utilized for the spin coating procedure. The application rate was approximately 7000 rpm for 1 min. of drying. The samples were conditioned in an air-conditioned room (2°C, 50% relative humidity) for 21 days prior to testing.

Tab. 2: Composition of polymeric coatings. (prepared in cooperation with Department of Paints and Organic Coatings, University of Pardubice)

Diatomite (SiO_2)	Tungstate (Fe_2WO_6)
SiO_2/PANI	Fe_2WO_6/PANI ϕ_i= 1%
SiO_2/PPDA	Fe_2WO_6/PANI ϕ_i= 15%
SiO_2/$ZnFe_2O_4$	Fe_2WO_6/PPDA
Wollastonite ($CaSiO_3$)	Molybdate ($Fe_2(MoO_4)_3$)
$CaSiO_3$/PANI	$Fe_2(MoO_4)_3$/PPy
$CaSiO_3$/PPDA	$Fe_2(MoO_4)_3$/PANI
$CaSiO_3$/$ZnFe_2O_4$	$Fe_2(MoO_4)_3$/PPDA

Reference sample - WorléeDur D 46

The coatings were prepared in cooperation with the University of Pardubice, Faculty of Chemical Technology, Institute of Chemistry and Technology of Macromolecular Materials, Department of Paints and Organic Coatings.

3.1.3 Preparation of nanostructured surfaces based on TiO_2

TiO_2 NTs were fabricated by the electrochemical anodization of Ti foil (Advent Research Materials, England) of 0.1 mm thickness (99.6% purity), as published previously (Moyen *et al.*, 2016; Luo, 2013). Briefly, NTs were obtained by a two-step anodization method, where, in the first step, a pre-patterned surface was obtained by electrochemical anodization of Ti foil in an ethylene glycol (EG) electrolyte (with 1M H_2O and 0.1M NH_4F) at 35 V for 2 h, followed by ultrasonication in deionized water (DI) water in order to remove the grown nanotubular layer. In the second step, electrochemical anodization was conducted using EG electrolyte containing 8 M water and 0.2 M hydrofluoric acid (40% HF solution). The anodization conditions are presented in Tab. 3. All reagents were purchased from Sigma–Aldrich (Germany) and used without further purification. The NTs were prepared in cooperation with Jožef Stefan Institute in Ljubljana, Slovenia.

Tab. 3: Anodization conditions for different TiO_2 NTs. (prepared in cooperation with Jožef Stefan Institute)

TiO_2 NTs diameter	Electrolyte	Potential	Anodization time
15 nm	EG + 8 M water + 0.2 M HF	10 V	2.5 h
50 nm	EG + 8 M water + 0.2 M HF	20 V	2.5 h
100 nm	EG + 8 M water + 0.2 M HF	58 V	2.5 h

3.2 Characterization of surface properties

3.2.1 Atomic force microscopy

Topographic changes of the polymeric surfaces after plasma treatment were monitored with AFM (Solver PRO, NT-MDT, Russia) in the tapping mode in air. The samples were scanned with a standard Si cantilever with a force constant of 22 $N.m^{-1}$ and at a resonance frequency of 325 kHz. The surface roughness has been measured on 1 x 1 μm^2 and 2 x 2 μm^2 AFM images, as this size of the area was the most representative for roughness measurements of samples. The surface roughness was expressed in the terms of average surface roughness (Ra) and it corresponded to the average height of the features at the surface. This test was performed in cooperation with Jožef Stefan Institute in Ljubljana, Slovenia.

3.2.2 Surface energy

Contact-angle data were obtained with a Surface Energy Evaluation System (SEE system) from Advex Instruments (Czech Republic). Deionized water, ethylene glycol, and diiodomethane have been used as testing liquids. The volume of droplets was set to 5 μL for all experiments to avoid errors associated with gravity acting on the sessile drop. Ten contact-angle readings were averaged to obtain one representative value. The free energy of the substrate surface was evaluated by the Lifshitz–van der Waals "acid-base" model (Wu, Giese and van Oss, 1995). Additionally, total surface energy (γtot) and its components, as well as disperse (γLW) and acid-base (polar) (γAB) components are reported.

The surface energy evaluation was performed in the laboratory of the Centre of Polymer Systems at TBU in Zlín.

3.2.3 Electrical conductivity

Turning to the conductivity of the PANI films, this was measured by the four-point Van der Pauw method. A programmable electrometer with an SMU Keithley 237 current source and a Keithley 2010 Digital Multimeter with a 2000 SCAN 10-channel scanner card (USA) were employed. Measurements were carried out at laboratory temperature.

The electrical conductivity evaluation was performed in the laboratory of the Centre of Polymer Systems at TBU in Zlín.

3.3 Evaluation of biological properties

3.3.1 Microbial strains used within the evaluation of biological properties

The strains of gram-positive and gram-negative biofilm-forming bacteria and filamentous fungi obtained from the Czech Collection of Microorganisms (CCM) were used for the research work of the doctoral thesis.

Biofilm-forming bacteria:

- *Staphylococcus aureus* CCM 2022
- *Staphylococcus aureus* CCM 3953
- *Staphylococcus epidermidis* CCM 4418
- *Staphylococcus epidermidis* CCM 7221
- *Pseudomonas aeruginosa* CCM 3955
- *Enterococcus faecalis* CCM 4224
- *Enterococcus faecalis* CCM 7000
- *Escherichia coli* CCM 3988
- *Klebsiella pneumoniae* CCM 4415

Filamentous fungi:

- *Aspergillus niger* CCM 8155
- *Gliocladium virens* CCM 8042
- *Paecilomyces variotii* CCM F-398
- *Trichoderma viridae* F-486

3.3.2 Bacterial biofilm formation

In the first step, the standard method using crystal violet dye was used for all of the tested samples. However, PANI owns such surface properties which cause that this method is not able to provide reliable results. Therefore, in the next step, the biofilm formation of bacteria was evaluated using a method measuring the ATP amount.

The bacterial biofilm formation test was performed with biofilm-positive bacterial strains listed in chapter 3.3.1. The bacteria were incubated at 30 °C and 37 °C (Memmert INE 600, Switzerland) for 24 h in accordance with the requirements of the individual strains. Bacteria were maintained on Nutrient agar No. 2 with glucose (HiMedia, India). The initial bacterial inocula were prepared by seeding the strains to the physiological solution with turbidity (τ=2) in adherence with the McFarland scale (McFarland and L'Engle, 1907) using a densitometer (Biosan, Latvia).

The process of quantifying the biofilm with bacteria followed a procedure described by Koutný *et al.* (Koutny *et al.*, 2006). In brief, 210 μL of Tryptone Soya Broth (Himedia, India) containing 20 μL of bacterial inocula was added to each well on a microtiter plate and incubated at 30 °C and 37 °C for 48 h, in accordance with the requirements of the individual strains. After incubation, the content of each well was carefully removed, rinsed with a physiological solution and dried in air. Determination of the level of ATP involved the use of the ATP Biomass Kit HS by Biotherma (Sweden). In order to release the cells from the surface, ultrapure water with Extractant B/S (BioThema, Sweden) at the ratio 1:1 was added to each well. The solution was mixed with ATP Reagent HS + diluent B solution (BioThema, Sweden) at the ratio 1:4, transferred to a cuvette, and then light emission ($I_{smp1/2}$) was measured in a luminometer (Turner BioSystems). It is followed by supplementation of the cuvette content with 10 μL of 100 nmol L^{-1} ATP Standard (BioThema, Sweden), which contained a known quantity of ATP, and light emission was repeatedly measured (Ismp+std). The amount of ATP (pmol) in the sample was calculated using the equation: $ATP_{smp} = I_{smp1} / (I_{smp+std} - I_{smp2})$, and the ATP level was subsequently expressed as the number of *E. coli* cells with help of calibration curve. The test was performed in quadruplicates. The statistical differences between the individual values were evaluated using Tukey's significant difference test.

The evaluation of bacterial biofilm formation was performed in the laboratories of the Centre of Polymer Systems at TBU in Zlín.

3.3.3 Fungal biofilm formation

The filamentous fungal strains, listed in chapter 3.3.1, were cultivated on malt-extract bouillon broth (MEBB, Himedia, India) solidified with agar (20 g L^{-1}), which was utilized as a nutrient-rich agar. Nutrient-poor agar contained the following components: $NaNO_3$ 1 g L^{-1}, $NH_4(SO_4)_2$ 1 g L^{-1}, K_2HPO_4 1 g L^{-1}, KCl 0.5 g $^{-1}$, $MgSO_4.7\ H_2O$ 0.5 g L^{-1}, $FeSO_4$ 0.01 g L^{-1}, soya peptone 0.1 g L^{-1}, agar 18 g L^{-1} and trace element solution 1 mL (Muchová *et al.*, 2009). Nutrient agar was inoculated with 0.1 mL of spore suspension of fungal strains, which was prepared as follows: individual mold was wiped three times by a loop in tubes with 3 mL of sterile saline solution. All samples including reference pure PP foil were sterilized using a UV light for 30 min, carefully placed on the surface of nutrient agar, and subsequently inoculated by application of spore suspension around the edges of the film and on the surface of the film by gently tapping of sterile cotton sticks. In the case of the research study listed in chapter 4.2, the mixed culture of filamentous fungal strains was used because of the small amount of tested samples.

All samples were incubated at 25 °C for 42 days, and images after 7, 14, 21, 28, 35 and 42 days of cultivation were taken. Fungal growth (on the edge) and biofilm formation (in the middle) on the polymeric surfaces was expressed as the percentage of area covered with fungal mycelium. Photographs of the samples were taken, onto which a grid was created (Fig. 28). The individual squares of the grid, which either did or did not contain fungal biofilm, were counted and percentages for growth and biofilm formation were determined. The growth of filamentous fungi on the nutrient-poor agar was also subsequently analyzed in the same way. All tests were performed in duplicates. The statistical differences between the individual values were evaluated using the Sign test.

The evaluation of fungal biofilm formation was performed in the laboratories of the Centre of Polymer Systems at TBU in Zlín, in cooperation with master student Kristýna Janů.

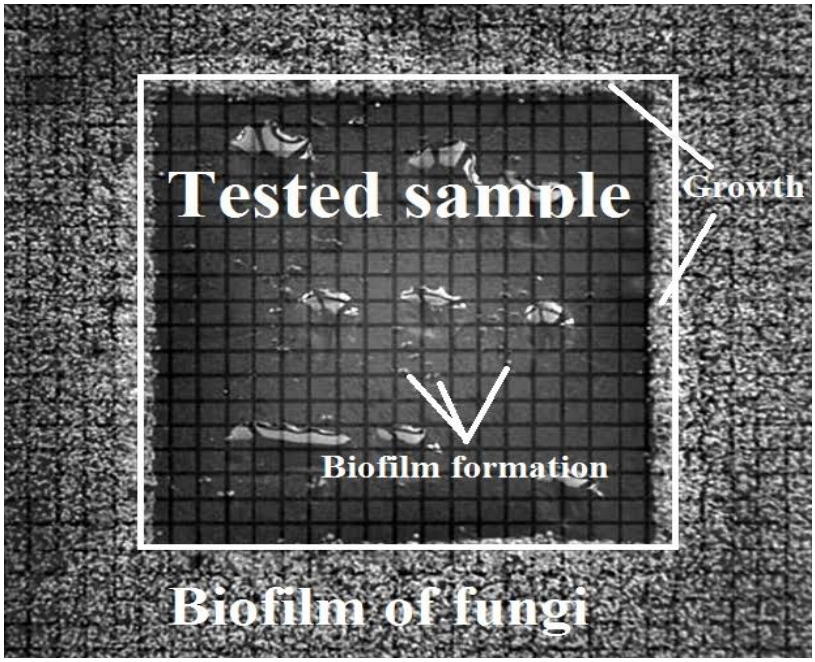


Fig. 28: Grid applied to evaluate biofilm formation and growth of fungi.
(The grid was prepared at the Centre of Polymer Systems)

3.3.4 Methods introduced to laboratory practice

3.3.4.1 Comet assay

Within the doctoral study, new methods were introduced to extend the competence of the biological laboratory in the Centre of Polymer Systems. This important step helped to contribute with new knowledge about tested material properties in the laboratories of the Centre of Polymer Systems. Comet assay and protein adsorption are well established methods for the biological characterization of materials for their application in biomedicine fields.

Comet assay was performed according to the procedure within the protocol of Olive and Banath (Olive and Banath, 2006) where is the whole methodology described in more detail. As materials were used following:

- Low-gelling-temperature agarose
- Phosphate buffered saline (PBS)
- Proteinase K
- 0.5 M Na_2EDTA
- Fluorescent DNA stain
- N1 solution: neutral lysis solution for double-strand-break detection
- N2 solution: neutral rinse and electrophoresis solution

The procedure is initiated by agarose preparation using two baths equilibrated at two different temperatures. The well dissolved solution of agarose was used for slide-precoating. Agarose was air-dried to a thin film. This methodology continued with sample preparation. A single-cell suspension was prepared using enzyme disaggregation or mechanical dissociation. It included a sample of untreated cells to confirm that background damage is low. As a positive control, the cells were exposed to the tested substance as is specified in the protocol. In our case, the diluted H_2O_2 solution was chosen because of the well-known cytotoxic activity on eukaryotic cells.

Lysis and electrophoresis were the next step of the assay described. After agarose was gelled, the slides were gently submerged in a covered plastic tube containing a N1 solution at 4 °C. The tubes were placed in an incubator at 37 °C overnight. After overnight lysis, the slides were removed and submerged in room temperature N2 rinse buffer for 30 min., repeated two more times. Further, the slides were submerged in a fresh N2 solution in an electrophoresis chamber that has been leveled and filled with a measured volume of buffer about 1-2 mm above the top surface of the agarose. Electrophoresis is conducted in solution N2 for 25 min. at 7 mA and 20 V. Then, the slides were placed in a staining solution containing 2.5 μg/ml of propidium iodide in distilled water for 20 min. This procedure is followed by a slide analysis using the fluorescence microscope and taking images of the cells. If DNA of the eukaryotic cell is damaged (it led to DNA double-strand breaks), it creates the tail which is called a comet.

3.3.4.2 Protein adsorption

Protein adsorption was utilized according to the instruction protocol included within the Micro BCA Protein Assay Kit (Thermo Scientific, USA) where is the whole methodology described in more detail. This kit contains followed chemicals:

- Micro BCA Reagent A (MA)
- Micro BCA Reagent B (MB)
- Micro BCA Reagent C (MC)
- Bovine Serum Albumin Standard Ampules (2 mg/mL).

Firstly, standard and working reagent (WR) were prepared using as a guide of the table in the protocol for the preparation of diluted albumin (BSA) standards. For the preparation of the Micro BCA WR, the formula for determining the total volume of WR was used. Subsequently, the samples for measurement were prepared as follows: a 1 ml droplet of the Dulbecco's minimum essential medium (DMEM) containing 10% bovine calf serum (BCS) was introduced onto each specimen by a pipet. After incubation for 4 h at 37 °C, the samples

were transferred to a new 24-well plate and washed thrice with 1000 ml PBS. 500 ml of 1% SDS solution was added to these wells and shaken for 1 h to detach proteins from the surfaces. The protein concentrations in the collected SDS solutions were determined using a MicroBCA protein assay kit – protein adsorption procedure. The absorbance was measured at or near 562nm on a plate reader. A standard curve was prepared by plotting the average Blank-corrected 562nm reading for each BSA standard vs. its concentration in μg/mL. The standard curve was used to determine the protein concentration of each unknown sample (taken from the instruction protocol included in the Micro BCA Protein Assay Kit).

4 RESULTS AND DISCUSSION

The results related to the presented topic are summarised in the following sections. Each section consists of the motivation of the research work, result and discussion and the outcome of the research work. Some of the presented results have been published in the journals with impact factor which are mentioned below.
The sections are entitled as follow:

- Biofilm formation on PANI based films
  - Biofilm formation on pure and modified PANI films (published in *Chemical Papers*, 2017, *71(2)*, pp. 505-512)
  - Biofilm formation on plasma modified PANI films (unpublished results)
- Biofilm formation on polymeric coatings (published in *International Journal of Polymeric Materials and Polymeric Biomaterials*, 2019, *68(4)*, pp. 152-159)
- Biofilm formation on nanostructured TiO_2 (published in *Materials Science and Engineering: C*, 2017, *77*, pp. 500-507)
- Methods introduced to laboratory practice
  - Comet assay and Protein adsorption

4.1 Biofilm formation on PANI based films

4.1.1 Biofilm formation on pure and modified PANI films

4.1.1.1 The motivation for the research work

For microorganisms, the process of biofilm formation is simply a survival strategy for adaptation in a new environment. Due to increasing tolerance and resistance to antibiotics and immune responses, microbial cells attached in the biofilm have become a threat to medical issues, as well as on industry. Generally, fungal and bacterial growth can disrupt material surfaces by causing corrosion, loss of function or accumulation of metallic and toxic compounds (Garrett, Bhakoo and Zhang, 2008). A major role in microbial attachment and adhesion is played by surface chemistry and topography (Gallarato *et al.*, 2017). However, to the knowledge of the authors, few studies have been published on the antimicrobial activity of PANI and its composites against bacteria (Gizdavic-Nikolaidis *et al.*, 2011; Kucekova *et al.*, 2013; Stejskal, 2013) or fungal strains (Binkauskiene, Lugauskas and Bukauskas, 2013; Chauhan *et al.*, 2010; Yehgambaram *et al.*, 2013). For instance, Kucekova *et al.* (Kucekova *et al.*, 2013) observed the antibacterial properties of PANI–silver films. Conducting PANI-S exhibited significant antibacterial properties against the bacterial strains *Escherichia coli* and *Staphylococcus aureus*, while the efficacy of the PANI-B was not expressive. After silver deposition, the activity of PANI-S was comparable with that of original PANI-S, whereas PANI-B with silver possessed different levels of antibacterial effect depending on the type of bacterial strain.

The present research is the first which explore the behavior of PANI films in contact with selected bacterial and fungal strains, thereby providing crucial data on biofilm formation and the behavior of microorganisms on PANI surfaces. The first determination of the antibiofilm activity of pristine and modified PANI films has been established using crystal violet dye. However, this method proved to be inappropriate because of distorted results. Crystal violet penetrated to bacterial cell and color the cell wall, moreover, it was absorbed into the structure of PANI films. This unwanted reaction between dye and films subsequently influenced the amount of crystal violet in every sample and distorted results which have been not usable for the next evaluation. Because of that, the methodology had to be changed to measuring ATP level using a luminometer which provided values being interesting and significant for this research work.

4.1.1.2 Results and discussion

Surface energy and conductivity

The evaluation of surface energy through contact angle measurement revealed a rise in the total surface energy (γtot) in each PANI film in comparison with the PP and PS references, as can be concluded from values for γLW and γAB (Tab. 4). Indeed, the hydrophilicity or hydrophobicity of the surface is an important characteristic that exerts a major impact on cell attachment and fungal and bacterial growth. The greatest value for surface energy was observed for PANI-B and PANI-PTA films. In contrast, the lowest value measured pertained to PANI-PAMPSA, which demonstrated the most notable inhibition of bacterial biofilm, as shown and discussed below.

Tab. 4: Total surface energy (γtot), the dispersive component of surface energy (γLW), and the acid-base component of surface energy (polar) (γAB) in mN m^{-1}. (measured in the laboratory of the Centre of Polymer Systems by Nikola Mikušová)

Surface	γtot	γLW	γAB
PP	29.2	28.6	0.6
PS	35.7	34.8	0.9
PANI-S	45.7	40.5	5.2
PANI-B	52.2	45.0	7.2
PANI-PTA	51.9	47.4	4.5
PANI-PAMPSA	42.2	39.7	2.5

As regards conductivity, standard PANI-S is a conducting polymer that has been intensively studied and characterized. Indeed, Stejskal & Sapurina (2005) reported its conductivity to be 2.6 S cm^{-1}. For deprotonated PANI-B film, conductivity decreased to 1.4×10^{-8} S cm^{-1}, and the polymer became virtually non-conducting (Stejskal and Gilbert, 2002). As for the conductivity of PANI-PAMPSA, a value of about 10^{-2} S cm^{-S} was reported for similar samples (Gribkova *et al.*, 2011). The conductivity of PANI-PTA films has not been published previously. The measured value of conductivity in the case of PANI-PTA within this research was 9.5 S cm^{-1}, i.e. similar to that of standard PANI-S.

Bacterial biofilm formation

The determination of bacterial biofilm formation showed interesting results. PANI surfaces, pure and modified, have reacted differently to resist bacterial adhesion as is seen in Fig. 29. PANI-S failed to exert any significant effect against biofilm formation. In fact, bacterial biofilm was found on this surface to the same extent as on the PS reference. Slightly lower values were merely observed for *S. aureus* CCM 3953 and *E. faecalis* CCM 7000. The surface of

PANI-B slightly inhibited the biofilm formation only in the case of *E. faecalis* CCM 7000 in comparison with the PS reference sample (from 0.69×10^7 cells on the reference to 0.01×10^7 cells on PANI-B). The other bacterial strains were not significantly reduced on the non-conducting surface. Marginally greater activity against the biofilm formation was observed in PANI-PTA compared with the reference. A higher number of cells in the biofilm on this surface was only detected for *E. coli* CCM 3988 when compared with the reference. The most pronounced effect against biofilm generation recorded for all tested surfaces pertained to the PANI-PAMPSA film. This modified surface was capable to inhibit the biofilm formation on all the bacterial strains, especially exhibited most developed biofilm observed for *E. faecalis* CCM 7000. Furthermore, it was found that PANI-PAMPSA showed higher antibiofilm activity against gram-positive bacteria, specifically against both strains of *Staphylococcus* and *Enterococcus*. It can be however concluded that gram-negative bacteria (*Escherichia*, *Klebsiella*, *Pseudomonas*) were able to grow on the surfaces of tested samples comparably as gram-positive bacteria. The differences are due to the individuality of bacterial strains, as in the case of PANI-PAMPSA where the significant difference was seen between *E. coli* and *K. pneumoniae*.

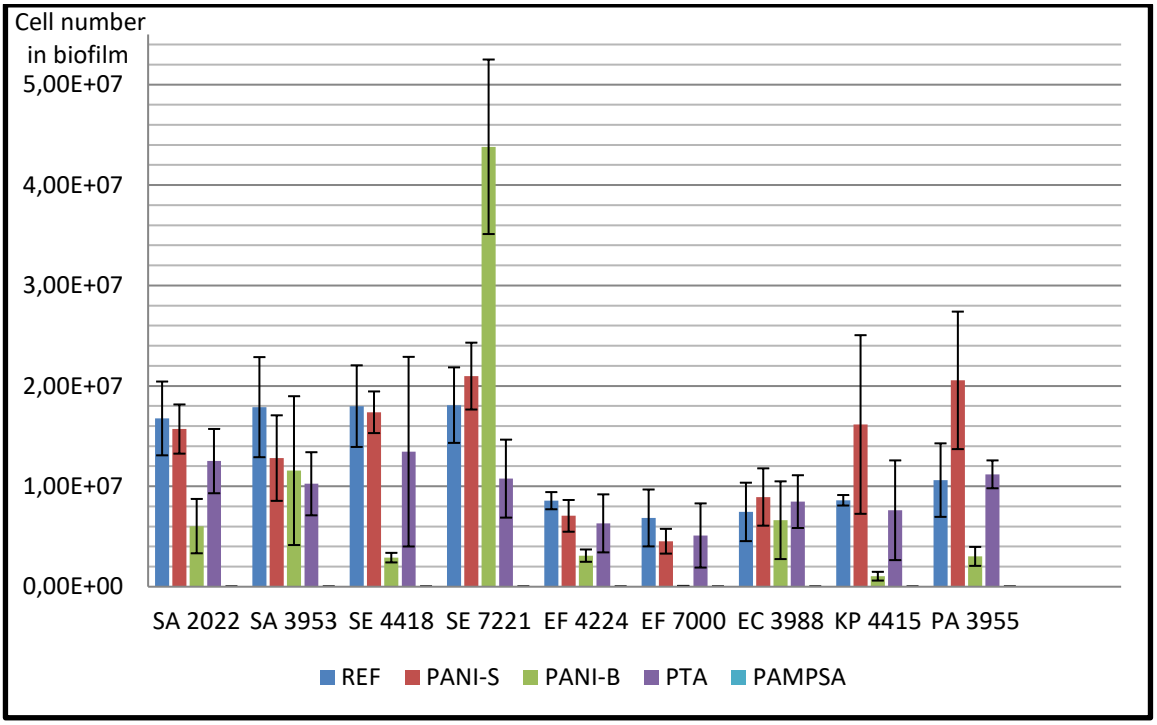


Fig. 29: Growth of bacterial biofilm formation expressed as an average number of cells with SD. (measured in the laboratory of Faculty of technology in TUB in Zlín by Nikola Mikušová)

The antibiofilm activity of the PANI-PAMPSA film stems from its surface energy being lower than other PANI films. PANI-PAMPSA has the most hydrophilic surface of the tested films. Generally, some strains of bacteria with

hydrophobic properties actually prefer more hydrophobic surfaces for their growth and vice versa, which is mainly influenced by surface charge. Therefore, bacteria were only able to form a biofilm on the surface of PANI-PAMPSA film to a very limited extent, the cause of which being its hydrophilicity (An, 2000). The study of De-la-Pinta *et al.* has also proved the advantage of hydrophilic surfaces in the fight against biofilm formation. They tested roughness and hydrophobicity of six different biomaterials to observe their influence on the biofilm formation of *E. coli*, *P. aeruginosa*, *S. epidermidis* and *C. albicans*, using CDC Biofilm Reactor. Biomaterials were used as follows: commercially available titanium (Ti), polycarbonate, silicone, borosilicate, Teflon and polyurethane disks. Teflon was evaluated as the roughest and most hydrophobic material with the highest growth of biofilm, whereas borosilicate with the least rough and hydrophobic surface and Ti had less developed biofilm on their surfaces (De-la-Pinta *et al.*, 2019).

The antibiofilm effect of PANI films against biofilm-positive bacteria has never been reported in detail in the literature. Studies have tended to concentrate on PANI in powder or colloidal forms or on PANI composites. Gizdavic-Nikolaidis *et al.* (Gizdavic-Nikolaidis *et al.*, 2011) have investigated the influence of functionalized PANI on the growth of bacterial cells. Their results showed that this polymer strongly inhibited the growth of wild strains of *E. coli*, *P. aeruginosa* and *S. aureus* and the authors determined their minimum inhibitory concentrations to be about 2.5 mg mL^{-1}. Indeed, in the follow-up study by Gizdavic-Nikolaidis *et al.* (Gizdavic-Nikolaidis *et al.*, 2012), the investigating of antimicrobial properties involved not only standard PANI, but also aniline oligomers extracted with acetone, for which inhibitory effects were also demonstrated for gram-negative and gram-positive bacteria and microscopic fungi. Kucekova *et al.* (Kucekova *et al.*, 2013) evaluated the antibacterial effect of pristine PANI-S and PANI-B films against *E. coli* and *S. aureus*. The study revealed that PANI-S exerted a significant antibacterial effect against both bacterial strains, while the efficacy of neat PANI-B remained only marginal.

Generally, the cell growth of *E. coli* and *S. aureus* were not significantly influenced or inhibited in our study compared to the aforementioned studies. Firstly, such variance in findings could be caused by the use of a different methodology for detecting bacterial biofilm formation. Secondly, the difference in the results obtained herein and the given studies might be due to the influence of the special biofilm-positive bacterial strains used in this research paper.

PANI-S is known to have a higher antibacterial effect compared with PANI-B, while in the present case PANI-B surface inhibited the growth of bacteria more than the surface of PANI-S. This fact could be explained by using biofilm formating bacteria compared to other studies which preferably use the commonly occurring bacteria.

Moreover, surfaces that contain ammonium salts or quaternary ammonium groups are generally known to have a damaging effect on both gram-positive and gram-negative bacteria due to the disruption of their cellular membranes (Tiller *et al.*, 2001). Positively charged nitrogen in this ammonium group interacts with the negatively charged head groups of acidic phospholipids in the bacterial cellular membrane. This act causes general perturbations in the lipids bilayers. The consequence of this reaction is that the bacterial cells release potassium ions which subsequently cause the loss of cell ability to undergo osmoregulation and their physiological properties as well (Gilbert and Moore, 2005).

Fungal biofilm formation

Fungal biofilm formation and mycelia growth were tested on both MEBB and nutrient-poor agar (see Fig. 30 and 31). After 42 days of cultivation, it was apparent that 100 % of the occurrence of fungal mycelia on the outer parts of the samples was observed in nearly all PANI surfaces. As for the biofilm growth on the inner parts of the samples, it can be concluded that the most intensive biofilm formation was recorded for PANI-PTA with *G. virens* (88 %), while the less intensive on PANI-B in *A. niger* (0 %) and PANI-PTA with *A. niger* (4 %). The surface of PANI-S showed the densest biofilm formation with fungi *G. virens* (72 %) and, similarly, with *P. variotii* (67 %). On the surface of PANI-B, the lowest biofilm formation was found in comparison with the other two types of tested samples. Generally, *G. virens* formed the densest biofilm, while the biofilm formation with *T. viridae* and *A. niger* was the lowest.

Initially, mycelia, which occurrences on the surfaces of samples, were visible at the borders, directly in contact with the agar media. It was observed that there was certain progress of mycelium growth toward the center of the samples after prolonged incubation or at the end of the test. This phenomenon led to the decision to perform an additional test to find out whether the films were able to serve as a nutritional source for fungal growth or if they merely functioned as an inert support for expanding mycelia from surrounding areas rich in nutrients. So as to assess the matter, experiments with *G. virens* and *P. variotii*, *A. niger*, *T. viridae* were conducted using nutrient-poor agar.

The tests were aimed to found if the PANI samples could serve as a nutrient source for the growth of used fungal strains. The highest degree of mycelium growth at the sample edge was observed on the surface of PANI-S with *G. virens* (100%). On the other hand, *A. niger* and *T. viridae* were not able to grow under such conditions on PANI-B and PANI-S materials. For PANI-PTA film, all four species of fungi were able to cover this surface significantly, although in the case of *G. virens* the extent equaled to 9 % only. In PANI-S and PANI-B, *G. virens* revealed the greatest degree of growth. As regards the biofilm on nutrient-poor agar, the most extensive formation was observed for *G. virens* in the PANI-S sample. Partial coverages were found for *G. virens* on

the PANI-B sample and for *T. viridae* on PANI-PTA material. PANI-PAMPSA film could not be tested on fungal biofilm formation because it was exfoliated from substrate material (PP foil).

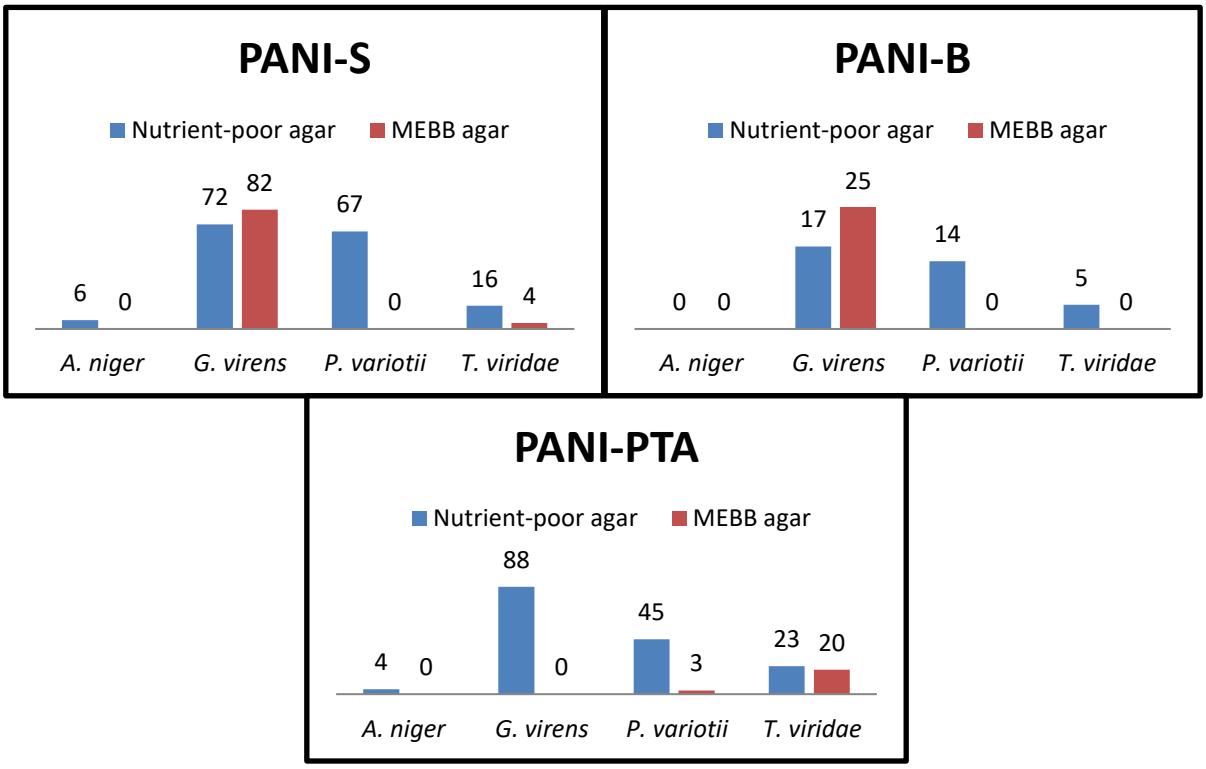


Fig. 30: Bar graphs represent biofilm formation of fungal strains expressed as % (according to the grid in Fig. 28, see chapter 3.3.2) on PANI films on nutrient-poor agar after 42 days of cultivation. The individual squares of the grid, which either did or did not contain fungal biofilm, were counted and percentages for growth and biofilm formation were determined.
(measured in the laboratory of the Centre of Polymer Systems in TUB in Zlín by Nikola Mikušová in cooperation with Kristýna Janů)

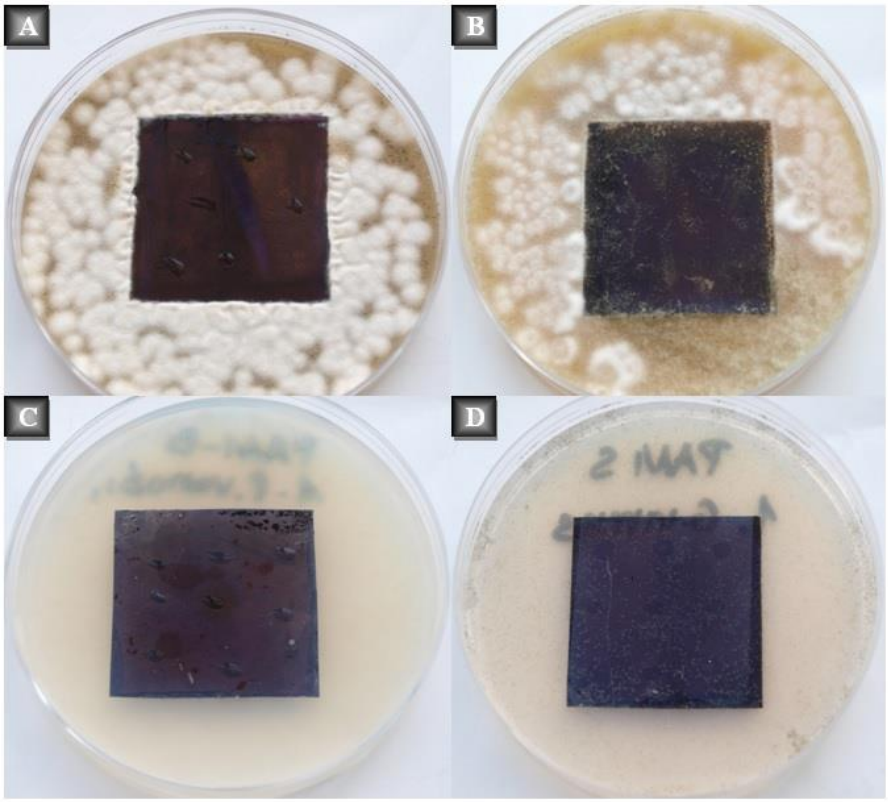


Fig. 31: Growth of filamentous fungi on various PANI surfaces after 42 days of cultivation on nutrient-rich and nutrient-poor agar. (A) *T. viridae* on PANI-B with nutrient-rich agar; (B) *G. virens* on PANI-PTA with nutrient-rich agar; (C) *P. variotii* on PANI-B with nutrient-poor agar; (D) *G. virens* on PANI-B with nutrient-poor agar. (The images were taken in the laboratory of the Centre of Polymer Systems in TUB in Zlín by Nikola Mikušová in cooperation with Kristýna Janů)

Comparing results from all the surfaces when utilizing nutrient-rich and nutrient-poor agar demonstrated that, in general, a higher percentage of the fungal growth was achieved with complete agar. It was the same in case of biofilm formation. Therefore, the employed strains needed complete agar to facilitate their growth and biofilm formation due to the utilization of nutrients, as nutrient-poor agar lacked sufficient nutrients for the same to take place. The use of nutrient-poor agar led to the releasing of mycelia occurrence on the tested samples in most cases, except for *G. virens* on the surface of PANI-S.

The filamentous fungi are not intensively studied compare to bacterial strains. However, Lugauskas *et al.* (Lugauskas, 2003) measured the various polymeric materials. The highest exposure of tested materials was in the case of filamentous fungi *A. niger* and *T. viridae*. Moreover, the results given herein correspond to findings by Binkauskienė *et al.* (Binkauskiene, Lugauskas and Bukauskas, 2013), who reported the growth of filamentous fungi on PANI-S surfaces. In their study, only nutrient-rich agar was applied, and the extent of

fungal growth observed was dependent on the given species, similarly as determined herein. Furthermore, fungal growth was influenced by electrochemical behavior, redox activity and oxalate impurities on the polymeric surface. Concerning the tested behavior of *A. niger* on PANI-S, the exposure of PANI after 7 days was already evaluated as 4.5 points from 5.

4.1.1.3 The outcome of the research work

Within this research, the knowledge about mutual interactions of polymeric surfaces and microbial attachment was significantly advanced. Pristine and modified PANI surfaces were subjected to the determination of their antibiofilm activity. Overall, the reprotonated surface of PANI-PAMPSA demonstrated antibiofilm activity against all tested strains of biofilm-positive bacteria. Whereas, the other tested PANI films did not show any significant antibiofilm activity. Probably thanks to the lowest level of surface energy in the case of PANI-PAMPSA film, microorganisms were not capable to attach its surface and form a biofilm. Concerning the anti-fungal activity, used filamentous fungi were able to form mycelia to a greater extent on PANI films, mainly on nutrient-rich agar compared to nutrient-poor agar. Nutrient-poor agar limited occurrence of mycelia in most cases. Fungi had not enough nutrients for their living and survival. These findings and surface properties have practical importance for various antimicrobial surface treatments.

This study was summarized into the manuscript which was subsequently submitted and published in the journal with impact factor as *Formation of bacterial and fungal biofilm on conducting polyaniline*, *Chemical Papers*, 2017, *71(2)*, pp. 505-512. Moreover, the study was already cited in three articles.

4.1.2 Biofilm formation on plasma modified PANI films

4.1.2.1 The motivation for the research work

Thanks to combined electronic and ionic conductivity the CPs are ideal materials for covering electrodes for biosensing (Sobolewski, Piwowarczyk and Fray, 2016) or for application where electrical stimulation is intended to be applied to the living cells or tissues (Bober *et al.*, 2015; Humpolicek *et al.*, 2015). Both the application in biosensors, as well as electrical stimulation of living subjects, assume the desired biointerface properties. As previously described by Rejmontová *et al.* (Rejmontová *et al.*, 2016), the ability of eukaryotic cells to adhere and grow on the PANI surface depends on the differences between the surface energy of PANI and respective cells. The surface energy is also crucial when the interaction with proteins of physiological fluids are considered. The surface energy can be modified by either chemical reaction (Queffélec *et al.*, 2012; Mikušová *et al.*, 2017) or by plasma treatment (Slepička *et al.*, 2012). Plasma provides diverse possibilities to refine a polymer surface, enabled by the adjustment of parameters like gas flows, power, pressure and treatment time. Due to the numerous ways, a plasma interacts with the polymer surface. The gas type (Ar, He, N_2, O_2) and plasma conditions must be adjusted on the polymer type to minimize degradation and aging effects (Hegemann, Brunner and Oehr, 2003; Sladek *et al.*, 2004). Plasma treatment also influences the wettability of materials, respectively increases hydrophilicity. Thus, it does not allow to most bacterial cells, which prefer less wettable, hydrophobic materials, to adhere and proliferate on the treated surfaces. In cooperation with Jožef Stefan Institute, in Ljubljana, Slovenia, PANI films treated by oxygen plasma were prepared and subsequently their properties tested.

4.1.2.2 Result and discussion

The films of PANI-S and PANI-B prepared according to the IUPAC procedure are the most representative form of the PANI. In the present research, those films were treated by radiofrequency coupled oxygen plasma and subsequently its wettability, morphology and chemical composition using AFM, X-ray photoelectron spectroscopy (XPS) and secondary ion mass spectrometry (SIMS) were investigated. Further, the conductivity of treated films was determined. To reveal the impact of plasma treatment on living organisms the study of bacterial biofilm formation and eukaryotic cell adhesion were performed.

For the reason that plasma treatment of surface changes both the surface chemistry and morphology, it can be classified as a chemical or physical modification. The surfaces treated by plasma offer excellent applications as biocompatible and antibacterial materials due to the combination of exceptional

surface stability and controlled chemical functionality (Bazaka *et al.*, 2011). However, plasma treatment also possesses certain disadvantages. Antimicrobial properties may be adversely affected because of undergoing of the functionalized surfaces further reactions. Plasma treatment suffering from several complications therefore needs to be carefully designed and carried out (Sambhy, Peterson and Sen, 2008).

The PANI films were treated by oxygen plasma in conditions which are described in chapter 3.1.1.2. The duration of treatment was set to 5 and 30 seconds. As it was mentioned above, the surface of a material is first which is in contact with prokaryotic and eukaryotic cells. Thus, the surface properties may influence their adhesion. The results from the investigation of surface properties and biological response of modified PANI films concerning the aging time are summarized in the tables (Tab. 5 and 6).

After plasma treatment, it was expected that the surface properties will stay stable for a long time and oxygen plasma will prolong the aging time of the surface properties of the tested material. Because material stability is important for medical fields. Conductivity generally depends on the oxidative state, reaction conditions, and form of PANI. From measuring conductivity it can be concluded that the value of untreated PANI-S (10 S cm^{-1}) was decreased after 5 s plasma treatment of glow regime approximately to 6 S cm^{-1} and the film was stable almost for the whole time of measuring of material aging. In the case of the afterglow regime, the conductivity of PANI-S was about 5 S cm^{-1} and during 21 days of testing, it was decreased even to 3.5 S cm^{-1} (Tab. 6). Thus, the glow regime seems to be more effective, the value was reduced only by one order. Plasma treated PANI-B film has stayed still nonconducting. The conductivity (10^{-8} S cm^{-1}) is too low to measure it using the SEE system device.

Tab. 5: Conductivity of PANI-S films after 5 s of oxygen plasma in the glow and afterglow regime (AG). (measured by Nikola Mikušová)

Conductivity G [S cm^{-1}]	PANI-S	
Day	Glow	AG
7	6.01	5.19
10	5.98	4.86
14	5.79	4.35
16	5.59	4.13
21	5.40	3.56

The water contact angle (WCA) was used to determine the wettability. Wettability has significantly increased. PANI-S films after treatment increased their hydrophilicity of the surface. However, it was found that 5 s plasma treatment is not practical and appropriate in this case. The surfaces were nonuniform and therefore it is necessary to lengthen the time of plasma treatment. On the contrary, PANI-B films showed excellent wettability for 10 days (Tab. 7). All tested films of PANI-B became fully wettable immediately after oxygen plasma. Wettability can positively contribute to preventing microbial adhesion or release their cells from the surface, or on the other hand to create an advantageous environment for eukaryotic cell adhesion and subsequent proliferation.

Tab. 6: Water contact angle of PANI-S and PANI-B films after 5 s and 30 s of oxygen plasma in the glow (G) and afterglow (AG) regime. WCA of untreated PANI films: PANI-S 68.24 °, PANI-B 73.86 °. (measured in the laboratory of Jožef Stefan Institute by Nikola Mikušová)

WCA [°]	PANI-S 5s		PANI-S 30s		PANI-B 5s		PANI-B 30s	
Day	Glow	AG	Glow	AG	Glow	AG	Glow	AG
1	29.93	15.95	0	11.05	0	0	0	0
2	32.11	28.91	0	24.27	0	0	0	0
3	36.84	32.83	19.12	24.99	0	0	0	0
4	38.25	38.54	25.11	26.41	46.58	0	0	18.99
7	41.69	39.70	28.80	32.62	40.07	0	0	19.14
10	46.63	41.89	31.12	35.78	29.00	0	0	31.23
14	47.25	50.62	42.25	36.36	40.99	29.24	26.94	36.54
21	36.70	41.23	29.90	42.31	42.04	33.25	29.01	39.18

The AFM of the tested surface showed that the morphology of PANI films is not homogenous. The values of Ra are only informative. However, how it is seen from micrographs in Fig. 32, the surface roughness was slightly decreased and the PANI films became smoother. So, plasma treatment has just gently changed the morphology. The roughness of surfaces stayed still high.

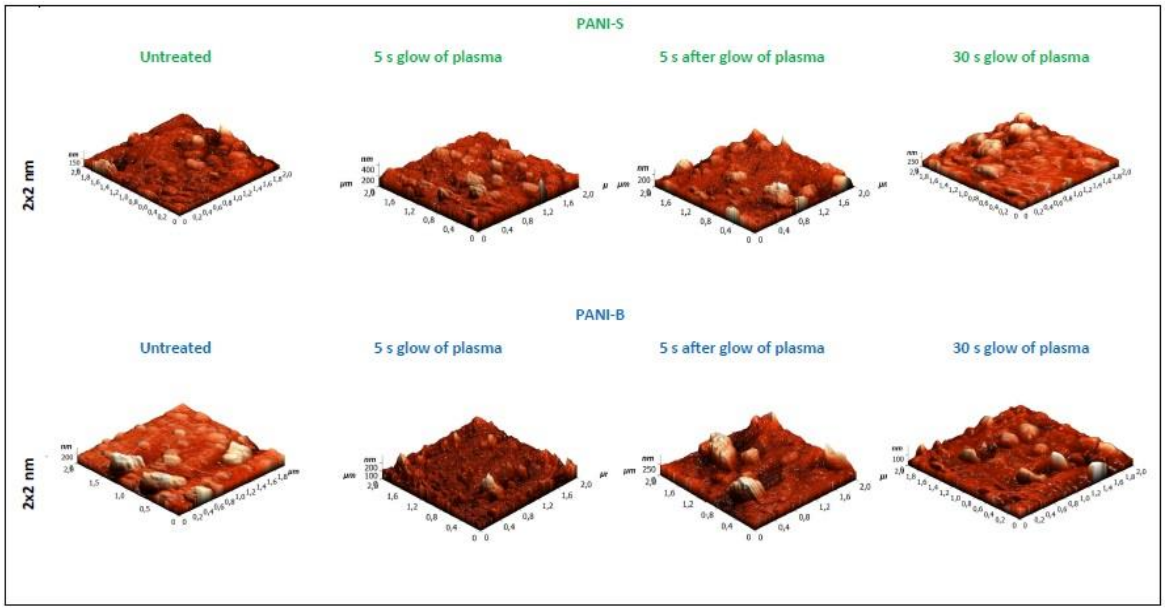


Fig. 32: AFM micrographs (2x2 nm²) of PANI films untreated and treated by oxygen plasma. Average surface roughness (Ra) with SD. (The images were taken in cooperation with the laboratory of Jožef Stefan Institute)

Subsequently, XPS of PANI films untreated and treated by oxygen plasma were performed and the obtained results confirmed the increased amount of oxygen onto the surface due to the oxygen plasma treatment (Tab. 7). The rest of the values were not significantly influenced, only the presence of atom C was slightly decreased after the plasma.

Tab. 7: X-ray photoelectron spectroscopy of PANI films untreated and treated by oxygen plasma. (measured in cooperation with the laboratory of Jožef Stefan Institute)

	Element	Atomic %	
		PANI-S	PANI-B
Untreated	C	75.3	77.4
	O	15.7	9.5
	N	4.3	8.9
	Si	3.5	4.3
	Cl	1.2	-
Treated	C	64.7	66.4
	O	24.5	25.4
	N	6.3	5.7
	Si	2.9	2.5
	Cl	1.2	-
	S	0.8	-

Concerning the biological response of plasma-treated surfaces, the growth of bacterial biofilm was observed, *S. aureus* representing gram-positive strain and *P. aeruginosa* representing gram-negative strain. Further, it was found that bacterial attachment was decreased compared to the reference (PP without PANI films) due to plasma treatment and probably high wettability of tested surfaces. However, the different times of plasma treatment did not show any significant influence on bacterial biofilm formation. The same finding was concluded for the aging time of PANI films. The effect of plasma treatment on eukaryotic cells (mouse embryonic fibroblast cell line ATCC CRL-1658 NIH/3T3, USA) did not prove any significant biocompatible activity (Fig. 33). Cell adhesion was almost to the same extent compared to treated samples.

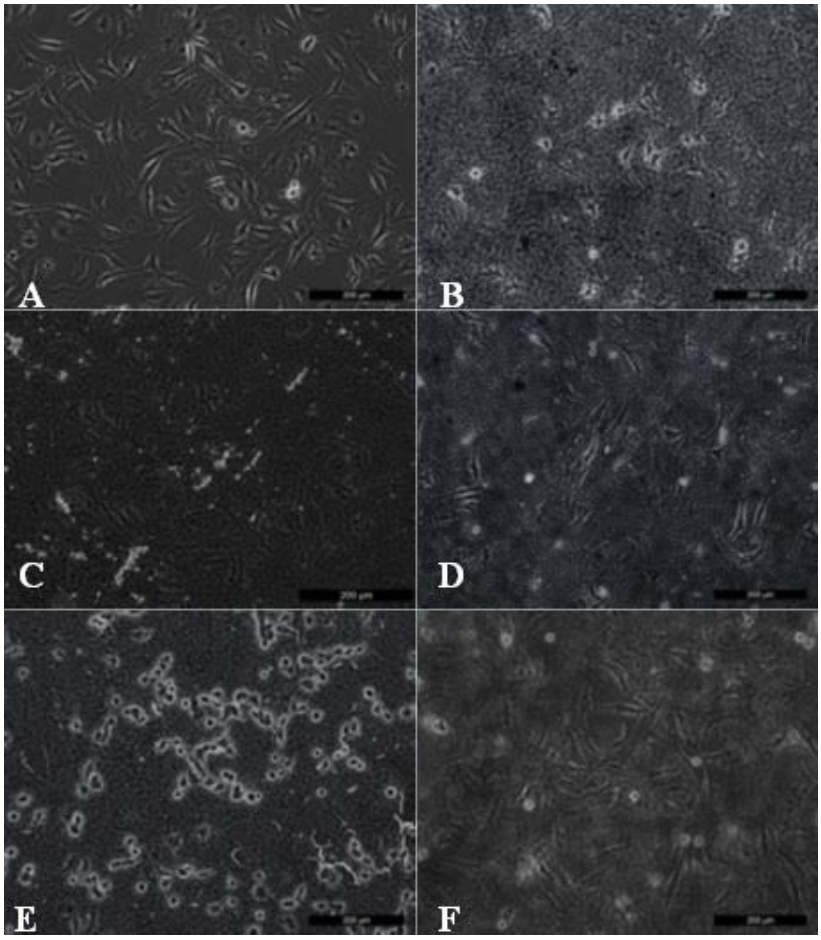


Fig. 33: Micrographs of adhesion of cell line NIH/3T3: A) reference (PS without PANI film), B) PANI-B 5 s, C) PANI-S, D) PANI-B, E) PANI-S 30 s, F) PANI-B 30 s. (Micrographs were taken in the laboratory of the Centre of Polymer Systems in TUB in Zlín by Nikola Mikušová)

Despite the fact, the plasma treatment has contributed to the improvement of the surface properties of PANI films, it is still necessary to upgrade the operation process of oxygen plasma, to choose the appropriate time of treatment

and to stabilize methodologies of measurements. However, observed wettability of the treated surface can be further developed for preparing biocompatible surfaces.

4.1.2.3 The outcome of the research work

The surface of microorganisms is usually hydrophobic, as well as the surfaces of CPs. These polymeric surfaces may be an appropriate substrate with antibiofilm activity. Due to this fact, the doctoral study was focused to obtain significant results which will be beneficial for broad scientific purposes. Within research, the oxygen plasma treatment was used for modification of polymeric surfaces to make them hydrophilic and to preclude the attachment of biofilm-forming bacterial cells.

4.2 Biofilm formation on polymeric coatings

4.2.1 The motivation for the research work

The main reason why the following study was introduced in our laboratories was the fact that biofilm formation on the surface can lead to the change of various surface properties (transparency, surface energy, conductivity, degradation of material resulting in a change of its composition). These undesirable changes in surface properties of polymeric coatings commit significant problems in many fields. Bacterial/fungal biofilm is also one of the crucial aspects of corrosion processes. The interesting properties of CPs are their ability to work as anti-corrosive agents (Kalendová *et al.*, 2008), for instance in the coating. It is assumed that the CPs passivate metals by forming a protective oxide on the surface of the metal, thereby reducing corrosion (Sitaram, Stoffer and O'Keefe, 1997).

The most extensively studied CPs are PANI, PPy, and poly(p-phenylenediamine), which have been applied with success in certain applications, e.g. active anti-corrosion agents for metallic materials by means of organic polymeric coatings (Armelin *et al.*, 2007; Deshpande *et al.*, 2012; Sathiyanarayanan *et al.*, 2005). Composite pigments combining the pigment (core) covered with a CP layer (shell) may eventually supersede toxic pigments containing lead or hexavalent chromium (Sambyal *et al.*, 2015). The advantages of such core/shell pigments with CP layers compare to CPs alone are as follows: a) increase in the number of contact sites in the polymeric coating; b) promotion of a synergistic effect exhibited by the CP and pigment core in anti-corrosion efficiency; and c) improvement in the physical properties of the polymeric coating. Polymeric coatings containing CPs solve the issue of providing anti-corrosion protection through organic coatings, these being referred to as "smart coatings".

Pigment diatomite (SiO_2) has good strength and abrasion resistance (Mleziva and Šňupárek). Wollastonite ($CaSiO_3$) improves thermal and mechanical properties and increasing of material durability (Mleziva and Šňupárek). Pigment zinc ferrite ($ZnFe_2O_4$) possesses high electromagnetic, excellent chemical stability and mechanical hardness (Naseri *et al.*, 2011). Further, tungstate (Fe_2WO_6) has an excellent heat resistance and high ductility (Pak, Bahgat and Paek, 2009). Whereas, pigment molybdate ($Fe_2(MoO_4)_3$) is a catalyst in formaldehyde synthesis (Oudghiri-Hassani, 2015).

A solvent-type epoxy ester resin was selected as a binder for the preparation of polymer coatings. The advantages of epoxy esters are excellent flexibility, long life, good adhesion, ease of handling, rapid air drying, good film toughness (Oil and Association, 1993). Moreover, it has excellent corrosion protection for aluminum and zinc pigments (Müller and Fischer, 2006). At present, new polymers are no longer developed for the preparation of anticorrosive coatings,

but the properties of already used polymers are modified. The properties can also be adjusted with additives (fillers, pigments, stabilizers, etc.) (Kalendová *et al.*, 2015; Mleziva and Šňupárek).

The ability of polymers is also to be as an "anti-biofouling" coating (Au *et al.*, 2013). Indeed, due to the combination of these properties, the CPs became to be an excellent candidate for application as an anti-corrosive coating together with the antibiofilm effect. Herein, the anti-biofouling properties and the surface energy of polymeric coatings including pigments modified by CPs were investigated to find the appropriate surface for the above-mentioned application of tested materials.

The coatings were prepared in cooperation with the University of Pardubice, Faculty of Chemical Technology, Institute of Chemistry and Technology of Macromolecular Materials, Department of Paints and Organic Coatings. At the laboratory of CPS, the biological tests and surface energy evaluation were performed.

4.2.2 Result and discussion

4.2.2.1 *Characterization of pigments*

The SEM analysis was used for evaluation of the surface and morphology of pigment particles and polymeric coatings separately because at low concentration of pigments the SEM analysis remained unchanged. The magnification of individual present figures depended on particle size given by its structure and type of use. The surface modification using CPs did not change the morphology or particle size and the pigments tended to form clumps, which had to be separated during dispersion in a polymeric binder. All of the tested coatings pigmented with 1% VCP had the same surface morphology by comparing each other. The pigments were applied in a polymeric binder by high dispersion, which forms a smooth and sintered film after application. In Fig. 34 there are seen the samples of surface modified and unmodified pigments.

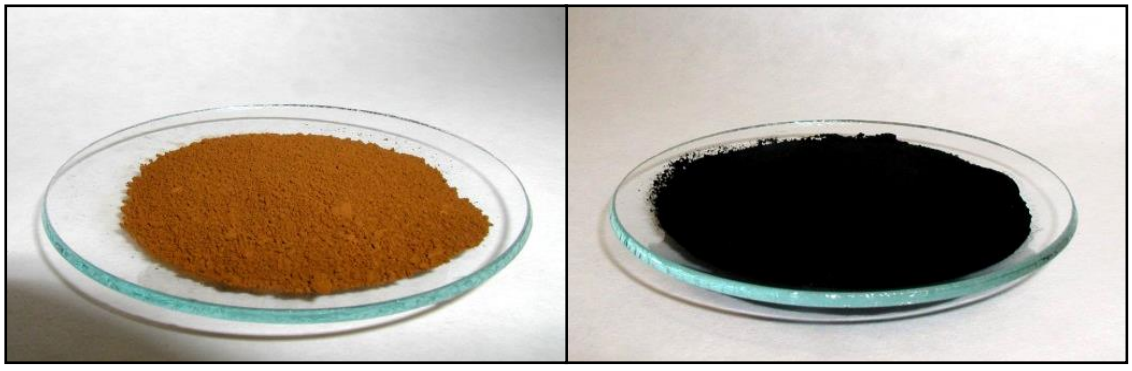


Fig. 34: The sample of surface unmodified pigment (left) and surface modified pigment of $ZnFe_2O_4$ using PANI. (Images were taken by Department of Paints and Organic Coatings, University of Pardubice)

The powder diffraction of tested pigments was drawn to know their exact content. Composite pigments (pigment / VP) contained an amorphous portion of the CP and a crystalline fraction of the pigment carrier. Pigment $Fe_2(MoO_4)_3$ /PANI contained a crystalline and amorphous fraction, $Fe_2(MoO_4)_3$ was the only present crystalline phase. In the case of the pigment $Fe_2(MoO_4)_3$/PPy, a crystalline and amorphous fraction is apparent from the diffraction diagram, $Fe_2(MoO_4)_3$ was present as the crystalline phase. The composite pigment Fe_2WO_6/PANI contained a crystalline and amorphous fraction. Pigment Fe_2WO_6 was the only present crystalline phase. The composite pigment Fe_2WO_6/PPy contained a crystalline and amorphous fraction and the main crystalline phase was Fe_2WO_6 and a small amount of hematite. $ZnWO_4$ was presented as a crystalline phase in this composite pigment. The composite pigment $ZnWO_4$/PPy contained a crystalline and amorphous fraction and the only crystalline phase presented in this pigment was $ZnWO_4$. The pigment $CaTiO_3$ contained crystalline phases: $CaTiO_3$, a small amount of rutile TiO_2, $Ca(OH)_2$, $CaCO_3$ and a small number of corundum Al_2O_3 (see Fig. 35). The pigment $Fe_2(MoO_4)_3$ contained little amorphous content, the major crystalline phase was $Fe_2(MoO_4)_3$, a small amount of MoO_3 was also present. The pigment Fe_2WO_6 contained the crystalline phases Fe_2WO_6 and WO_3.

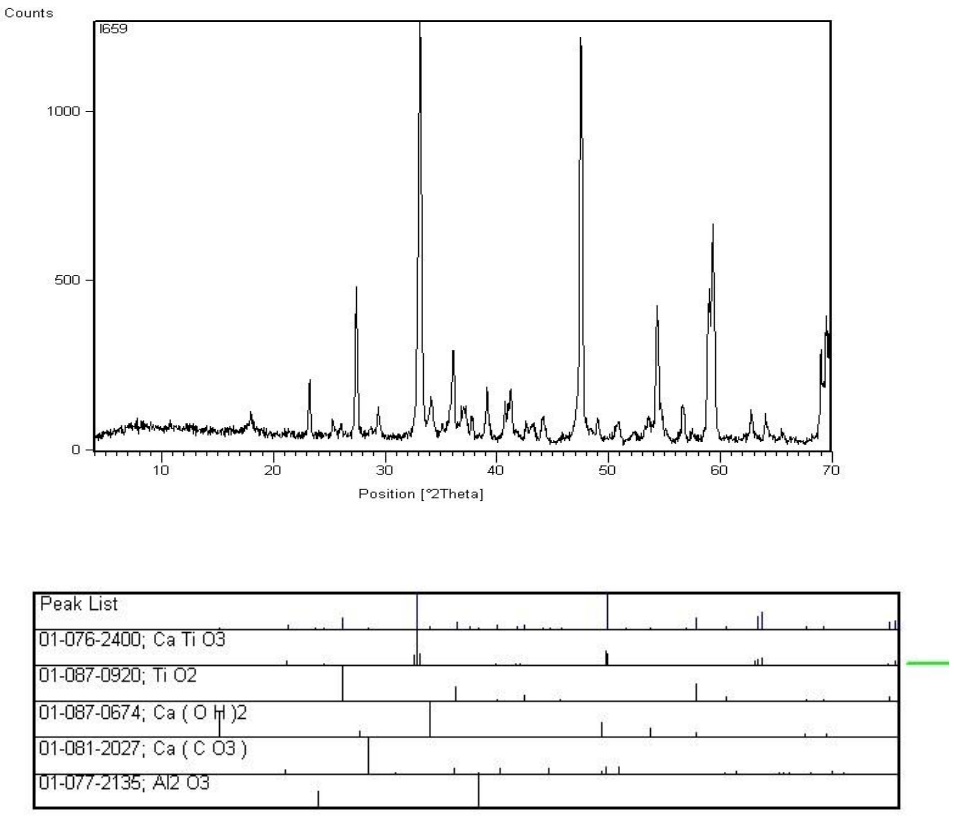


Fig. 35: The diffractogram of pigment $CaTiO_3$. (measured by Department of Paints and Organic Coatings, University of Pardubice)

4.2.2.2 *Surface energy*

As was described previously in the theoretical part of doctoral study, the formation of biofilm is influenced by numerous effects. Surface energy plays, in this case, a very crucial role. Based on obtained results, it obvious that pigmentation increased the surface energy of polymeric coatings. This fact can be supported by the claim that CPs and some pigments extend the drying time of polymeric coatings. Their use moreover makes a coating more plastic-like and decreases the hardness of the surface. If pigments are treated with CPs, the effects of the individual components are synergistic. Herein, surface treatment of SiO_2 with a CP was accompanied by a rise in surface energy, whereas surface modification of $CaSiO_3$ and Fe_2WO_6 brought about a slight drop in surface energy. Pigments differ in their acid-base and morphological properties, while $CaSiO_3$ is basic and its particles are clearly needle-shaped. Especially, in contrast with SiO_2, which is neutral to slightly acid with irregular particles. Pigments based on molybdenum and tungsten exhibit relatively regular-shaped particles. Modifying the surface of the pigments with CPs failed to alter the shape of the particles appreciably. In actual fact, the pigments largely become more porous, showing a complex surface and exhibiting binding sorption properties.

The differences in surface energy of each coating pertain to the nature of the pigment (chemical composition, particle shape), as well as to the presence of the CP. The effect of the CP and of the pigment on surface energy cannot be directly inferred from the value found for any individual concentration. The highest values for surface energy of the polymeric coatings were primarily observed in samples where the pigment surface had been modified with PPDA, except for the sample $CaSiO_3$/PPDA. The lowest surface energy was observed for the reference material WorléeDur D 46 (the epoxy-ester resin-based film itself with no added pigment).

4.2.2.3 Bacterial biofilm

The antibiofilm effect of the tested coating was described using two biofilm-forming bacteria species. The number of bacterial cells and related biofilm formation on the tested surfaces were affected by the composition of the polymeric coating. Generally, a methodology using ATP measurement did not show any significant differences or decreasing of bacterial adhesion. Only the weakest biofilm formation was observed on surfaces containing the pigment $Fe_2(MoO_4)_3$, especially in modification with PPy in the case of *P. aeruginosa* or modification with PPDA in case of *B. cereus*. Polymeric coatings with modified $CaSiO_3$ also reduced biofilm formation with regard to *P. aureginosa*, especially in the case of $CaSiO_3$/ $ZnFe_2O_4$.

Although, the biofilm formation on SiO_2/PANI was remarkable as the growth of both bacterial strains was significantly higher (by two orders of magnitude) compare to all other samples. The differences between SiO_2 and $CaSiO_3$

pigments from a chemical point of view are primarily in their pH: while SiO_2 is of medium acidity, $CaSiO_3$ is strongly basic. Their particle shapes were appreciably different as well. SiO_2, which possesses porous particles of a large, specific surface area, exerts a greater effect on the surface of the polymeric coatings; moreover, a layer of PANI is deposited in a stronger and more porous layer owing to the suitable properties of the pigment (Veselý, Kalendová and Němec, 2010; Liang, Li and Ruan, 2015). The film's surface produced could also be rougher and more complex. The resulting SiO_2/PANI particles exhibit a larger, specific surface area and more porous structure, also in comparison with other surface-modified SiO_2 pigments. The SiO_2 pigment coated with PPDA also demonstrated slightly more extensive biofilm formation, although this was not significantly higher than the other pigment types.

Paper of Guo *et al.* (Guo and Ma, 2018) is focused on polymers for combating biosensors. The main purpose was the summarizing up the progressive status of polymeric coatings used for combating microbial corrosion. CPs are now regarded as the most promising replacement of chromate ion coatings for the control of corrosion due to their environmental stability, high conductivity, and unique redox mechanisms. The research group of Kim *et al.* (Kim *et al.*, 2018a) had efforts to develop facile and effective antibacterial coatings on various oxide substrates. As a coating material, a random copolymer, abbreviated as poly(TMSMA-r-PEGMA), was synthesized. Polymeric self-assembled monolayers of poly(TMSMA-r-PEGMA) were formed on various inorganic oxide substrates, including silicon oxide, TiO_2, aluminum oxide, and glass, via the simple dip-coating process. It was subsequently characterized by ellipsometry, contact angle measurements, and X-ray photoelectron spectroscopy. The results showed that compared to the uncoated bare substrates, the substrates coated with polymeric films showed no bacterial adhesion, demonstrating the excellent antibacterial effect. All substrates became hydrophilic with the static water angle converging to 40°-42°, enabling them to maintain the hydration layer on the top surface.

4.2.2.4 Filamentous fungi biofilm

Because of the limitation of studies that are focused on fungal biofilm, it was decided that within this research the interaction of selected fungal strains and polymeric coating modified with pigments will be tested (Fig. 36). The observed results contribute more information to knowledge about the anti-fungal activity of CPs. Herein, the authors mimicked real conditions by inoculating a mixed culture of four fungal species. It was tested if the fungi overgrowth on the polymeric coating from the surrounding agar (from which can fungi draw nutrients) or if they are even able to form a biofilm without contact of filaments with agar. Therefore, cultivation on complete (MEBB) and incomplete agar was tested.

On MEBB, it was determined that the greatest degree of growth occurred in the case of $CaSiO_3$/PPDA, while the least growth was seen on the surface with the $CaSiO_3$. More intensive biofilm formation on MEBB – compared with the reference sample – was observed almost on all surfaces, except SiO_2, SiO_2/PANI, and $SiO_2/ZnFe_2O_4$. The effect of SiO_2 is not connected to the surface energy of the final material as they were similar to the reference.

The test performed on incomplete agar should reveal if the fungi were able to use any of the tested materials as a source of nutrients. Based on the results obtained in this work we can conclude that biofilm overgrowth and formation on all tested polymeric coatings were lower than on reference. The fungi were not therefore able to use the polymeric coatings as a source of nutrients. The lowest extent of biofilm formation was observed in the sample $SiO_2/ZnFe_2O_4$.

The polymeric coatings have not been tested yet on biofilm formation. The published studies are rather focused on testing of physicochemical features (Kalendová *et al.*, 2015). Fateixa *et al.* (Fateixa *et al.*, 2009) within their research found the nanocomposite of SiO_2 (the carrier of the active antifungal agent) in combination with Ag_2S showed considerable antifungal activity on *A. niger*. Further, the influence of the growth of *A. niger* on material corrosion was evaluated (Binkauskiene, Lugauskas and Bukauskas, 2013). The corrosion was accelerated in the case of tested materials which were coated using zinc. However, aluminum coatings had a slowing effect on corrosion. The polymeric materials containing various additives and homogenous materials were the main subject of the research work of Lugauskas *et al.* (Lugauskas, 2003). They found that homogeneous foils were not easily colonized by filamentous fungi as polymeric coatings containing different additives. Because the tested fungal strains used more compounds from inhomogeneous coatings as nutrients. It can be compared to polymeric coatings on the nutrient-poor agar where the lack of nutrients forced the fungi to draw nutrients from the polymeric coatings and not from the agar.

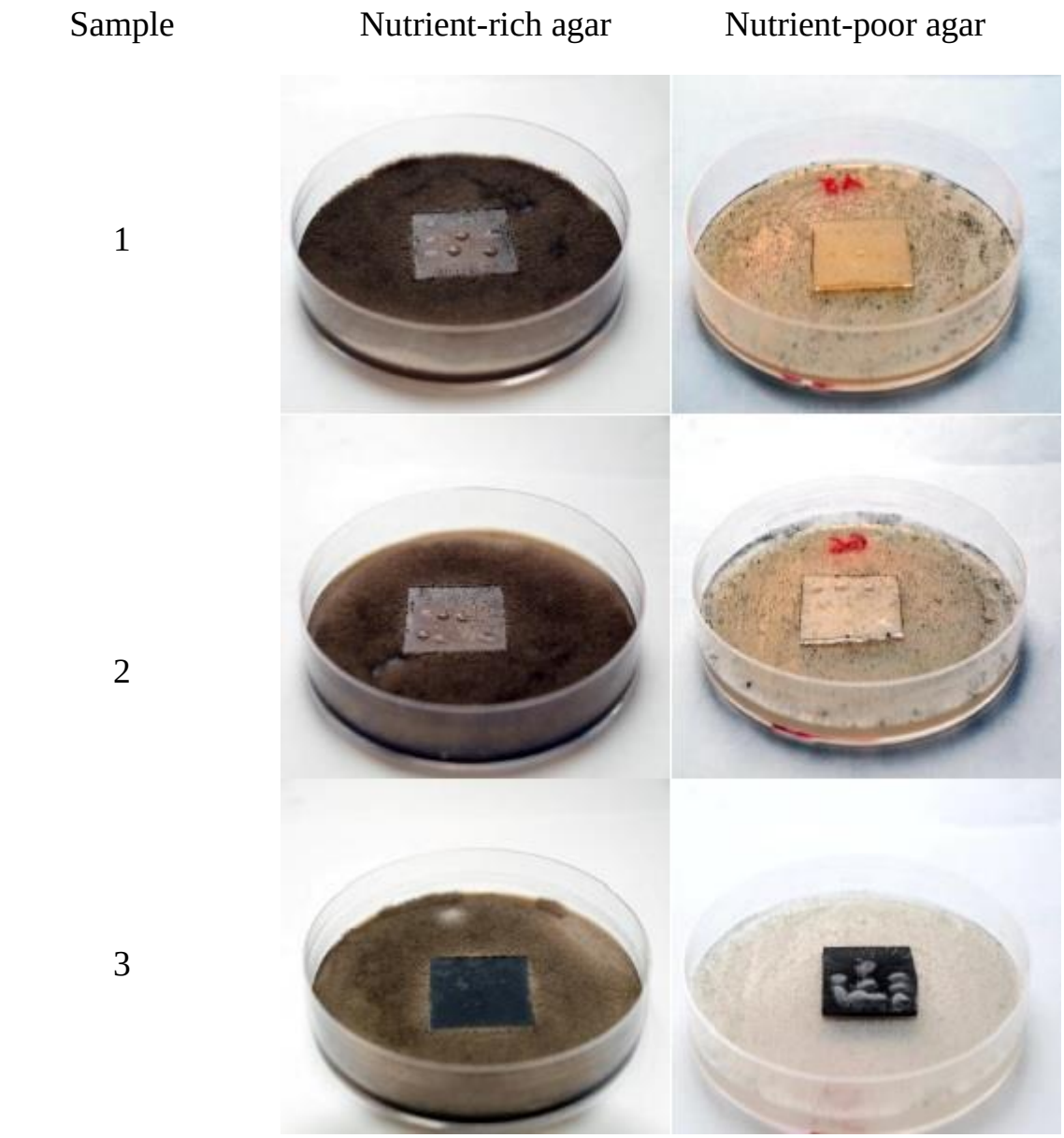


Fig. 36: Growth of filamentous fungi on various polymeric coatings after 42 days of cultivation on nutrient-rich and nutrient-poor agar; sample 1 – SiO_2, 2 – $CaSiO_3$/PPDA, 3 – PANI/Fe_2WO_6 VCP=15 %. (Images were taken in the laboratory of the Centre of Polymer Systems in TUB in Zlín by Nikola Mikušová in cooperation with Kristýna Janů)

4.2.3 The outcome of the research work

Tested anticorrosion coatings and their composition can lead to the formulation of a new anti-biofouling surface using in many fields of industry. The application of surface coatings is one of the most frequently used methods for the fabrication of antibacterial surfaces. Moreover, it expands the information about CPs and their modification with pigments. The biofilm-forming bacterial and fungal strains were utilized to detect their ability to grow on modified polymeric films. Additionally, the surface energy was determined. Results contribute to the understanding of the relation between CPs, polymeric coatings, their modification and microbial biofilms, especially filamentous fungi which was not previously studied.

In conclusion, the current success of the antibacterial activity of CPs offers great potential for mitigating and preventing biocorrosion in the future.

This study was summarized into the manuscript which was subsequently submitted and published in the journal with impact factor as *The effect of the composition of a polymeric coating on the biofilm formation of bacteria and filamentous fungi, International Journal of Polymeric Materials and Polymeric Biomaterials*, 2019, *68(4)*, pp. 152-159. Moreover, the study was already cited in two articles.

4.3 Biofilm formation on nanostructured TiO_2

4.3.1 The motivation for the research work

Next cooperation with Jožef Stefan Institut (Slovenia) was focused not on polymeric surfaces, but on TiO_2 NTs differing in their nanostructure. Ti and its alloys are commonly employed in medical implants, but progress in nanotechnologies together with new findings indicating the importance of nanoscale morphology on biofilm formation. Thus, biofilm formation, which is highly relevant for all implantable materials, was studied. Due to the rise of antibiotic-resistant bacterial strains, it is also of primary importance to inhibiting the growth of biofilms on implantable materials by influencing their surface properties. Various studies have already reported that nanotubular features could reduce bacterial adhesion (Narendrakumar *et al.*, 2015).

The NTs were prepared by Jožef Stefan Institute. In the laboratory of the Centre Polymer Systems, the biological evaluation was performed.

4.3.2 Result and discussion

4.3.2.1 Nanoscale morphology and surface chemistry of TiO_2 NTs

The surface properties of both Ti foil and TiO_2 NTs were characterized in terms of surface morphology (using SEM and AFM techniques), wettability, and surface chemistry (using XPS).

The analysis of surface morphology by SEM and AFM techniques revealed that pristine Ti foil had no special morphological features, while a uniform nanotubular structure was observed for electrochemical anodized TiO_2 surfaces (Fig. 37). From SEM images it can easily be observed that TiO_2 NTs were evenly distributed on the surface and that anodization potentials of 10 V, 20 V, and 58 V led to the formation of TiO_2 NTs of 15, 50 and 100 nm in diameter, respectively (Fig. 37). According to the results of AFM, Ti foil was not completely flat, as the average surface roughness measured on a 3x3 μm^2 area was about 19.3 nm. In the case of NTs, the calculated average surface roughness increased with NTs diameter, from about 11.7 nm for 15 nm NTs, to about 18.6 and 27.9 nm for 50 and 100 nm NTs, respectively. Although penetration of the AFM tip inside the hollow NTs interior was limited, due to the tip radius and the size of the NTs, the calculated roughness values and images obtained to provide additional information about the 3D structures of the NTs, these having the specific ability to interact with different cell types. Based on the previously published work it can conclude that the NTs have about 1 to 4 um in height and are stable on the surface (Kulkarni *et al.*, 2016).

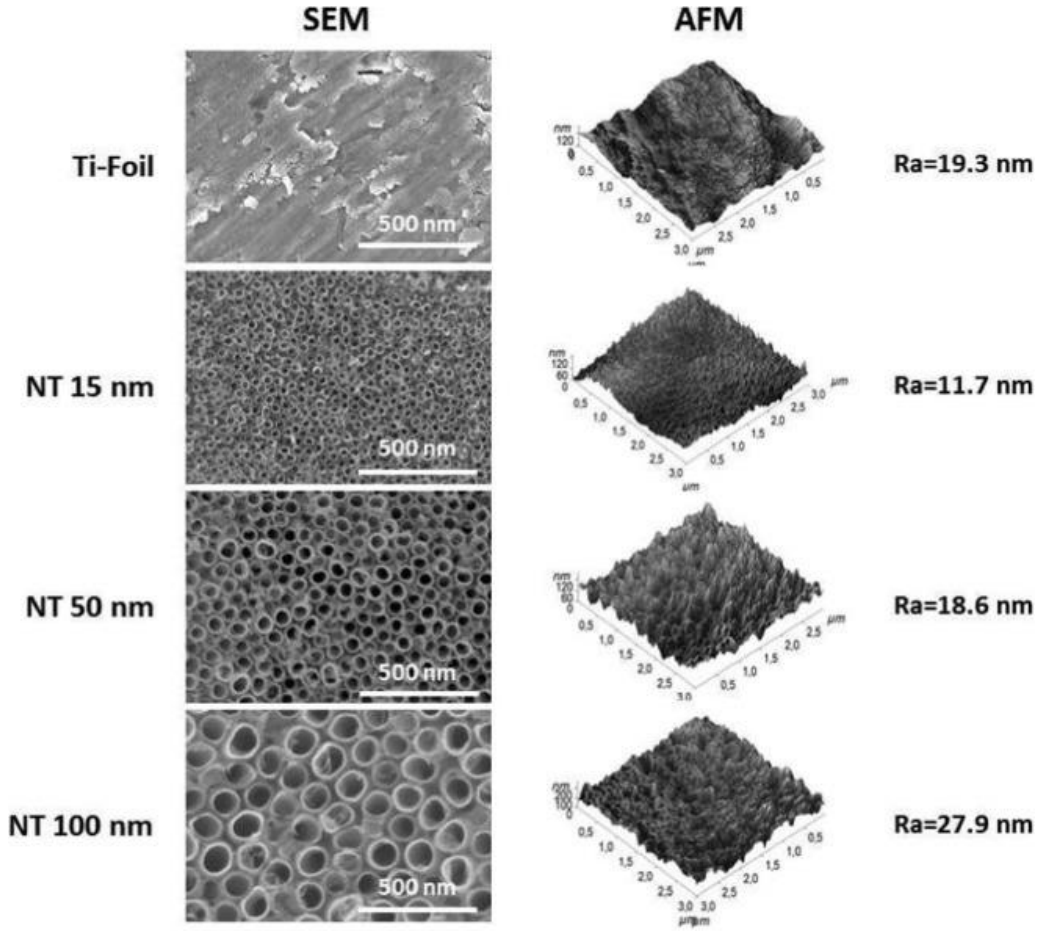


Fig. 37: Surface morphology of Ti foil and TiO_2 NTs determined from images taken by SEM and AFM. (measured by Jožef Stefan Institute)

Another important aspect of a biomaterial surface is its wettability, which could, together with other surface properties, influence on the biological response. WCA measurements conducted on Ti foil and freshly fabricated TiO_2 NTs indicate that Ti foil is poorly wettable (a water contact angle of about 780), while TiO_2 NTs are all hydrophilic (a water contact angle of less than 50). However, it should be noted that fabricated TiO_2 NT surfaces tend to age if exposed to the atmosphere and become hydrophobic. Thus, to ensure the hydrophilic character of the surfaces, all biological experiments were conducted one week after fabrication.

The chemical compositions of Ti foil and TiO_2 NTs of different diameters were determined from the XPS survey spectra (Tab. 8). No significant differences in chemical composition were observed between different diameter NTs. Moreover, similar surface chemistry was observed for pristine Ti foil. The only difference was in the presence of fluorine on the surface. The fluorine content was about 3-5 % and was ascribed to the remains of the electrolyte used for electrochemical anodization. In figure 38A, the survey spectra for 100 nm NTs are presented. It can clearly be seen that only C, O, Ti, N and F atoms are

present on the surface. Similar survey spectra were also recorded for the other NT diameters as well as for the Ti foil. From the high-resolution spectra of C 1s, O 1s, and Ti 2p, which are presented in figures 38B-38D, no significant differences in peaks among different NT diameters and between NTs and Ti foil were observed. The C 1s peak has a main peak at 284.8 eV, corresponding to C-C and C-H groups, and a small shoulder peak appearing at 288.9 eV, which corresponds to C=O bonds (Fig. 38B). In the case of the O 1s high-resolution scan, a primary peak at a binding energy of about 530.2 eV can be seen, which corresponds to the TiO_2 component (Fig. 38C). In the case of the Ti 2p peak, a doublet peak with maxima at 458.7 eV and 464.5 eV was observed, which is typical for the TiO_2 component (Fig. 38D). Differences in high-resolution peaks among different NTs diameters were not observed, indicating that the binding of C, O, and Ti is similar for all NTs.

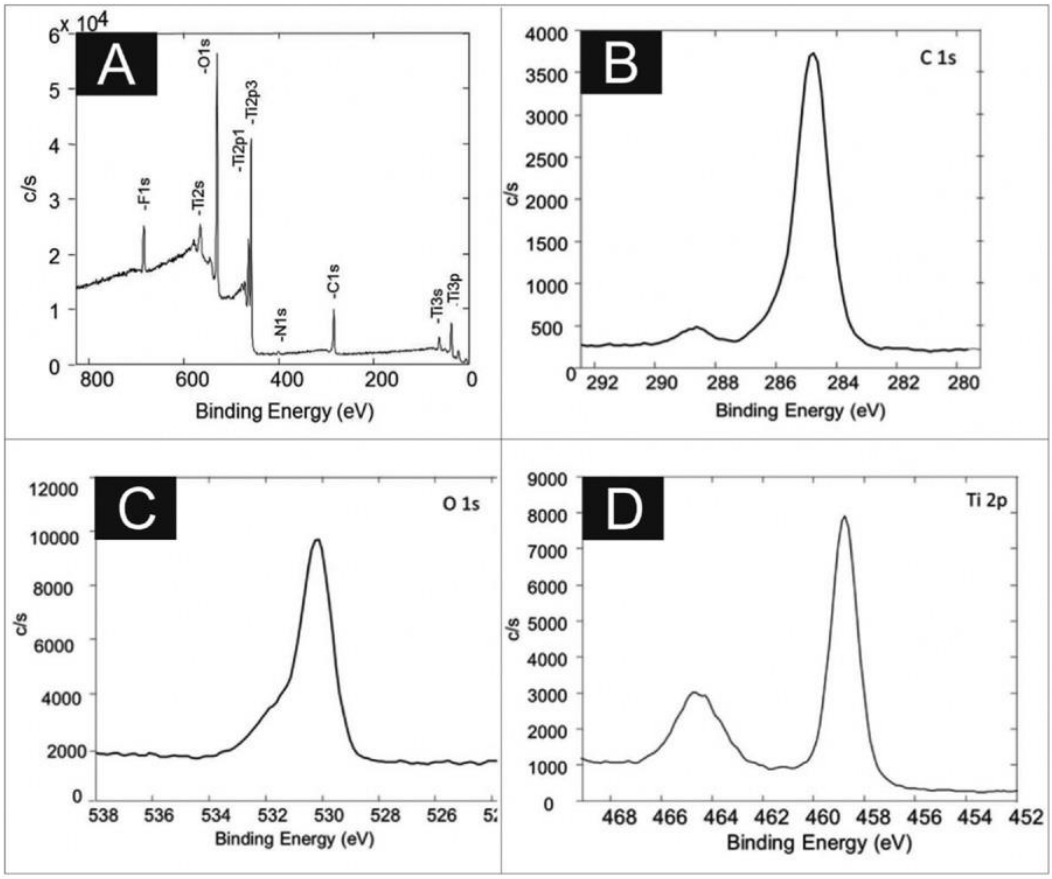


Fig. 38: Surface chemistry determined by XPS analysis; (A) XPS survey spectra for TiO_2 NTs of 100 nm in diameter, and the high-resolution scan of TiO_2 NTs of 100 nm in diameter for (B) C 1s, (C) O 1s, and (D) Ti 2p peaks. (measured by Jožef Stefan Institute)

Tab. 8: Surface composition of pristine Ti-foil and freshly prepared NTs of 15, 50, and 100 nm in diameter obtained with XPS. (measured by Jožef Stefan Institute)

	Atomic %				
Material	C	O	Ti	N	F
Ti-foil	38.3	41.2	18.0	2.5	0.0
15 nm	39.2	41.3	15.1	1.3	3.1
50 nm	36.2	42.7	16.1	0.8	4.2
100 nm	37.6	39.9	16.1	1	5.4

4.3.2.2 Bacterial biofilm

The nanostructure of material provides crucial factors for bacteria to attach to it. Therefore, the interaction of any biomaterial with eukaryotic cells, tissues or the immune system begins on the surface of a material and influences the biocompatibility of this whole process. Ti and its alloys are commonly employed for medical implants. However, progress in nanotechnologies together with new findings indicating the importance of nanoscale morphology on cell behavior opens the door for the application of Ti nanostructured surfaces.

In the case of nosocomial infections and implants and other medical treatment, the interactions with bacterial biofilm-forming species are highly relevant. Within this research two bacteria, *B. cereus* and *P. aeruginosa*, representing both gram-positive and gram-negative biofilm-forming bacteria, were studied. Both bacteria formed their biofilms with slightly higher amounts on TiO_2 NTs compared to Ti foil (Tab. 9). In addition, slightly higher amounts of formed biofilm were observed for *B. cereus* compared to *P. aeruginosa*.

The study has been extended to the determination of eukaryotic cell behavior. Three different cell lineages – mesenchymal stem cells, embryonic stem cells, and cardiomyocytes – were seeded on nanostructured surfaces of Ti. Especially, differences in their behavior were observed. Results showed that mesenchymal and embryonic stem cells were able to adhere and grow on the tested surfaces. Whereas the adhesion of cardiomyocytes was minimal. Moreover, a correlation between the NT diameter and cell behavior was observed. The 100 nm NTs appear to present a crucial diameter for, which NTs, critically influence mesenchymal stem cells behavior, while the 15 nm NTs appear to present a critical diameter for embryonic stem cells.

Overall, it can be concluded that bacterial strains are able to grow on various surfaces to the same extent in comparison with eukaryotic cells where significant growth difference for individual cell lines is observed.

Tab. 9: Biofilm formation expressed as the number of bacterial cells on the area 28.3 mm^2 after 48 hours incubation. (measured in the laboratory of the Faculty of technology in TUB in Zlín by Nikola Mikušová)

Sample	*Bacillus cereus*	*Pseudomonas aeruginosa*
Ti foil	1.24 $x10^6$	2.52 $x10^6$
15 nm	66.5 $x10^6$	7.10 $x10^6$
50 nm	16.7 $x10^6$	12.5 $x10^6$
100 nm	52.5 $x10^6$	2.42 $x10^6$

Although, Ti NTs are rather investigated for antibacterial activity compare to antibiofilm effect, the study of Lin *et al.* (Lin *et al.*, 2016) was focused on the examination of antibiofilm-forming properties of various diameter quaternized chitosan-loaded Ti NTs using standard strains methicillin-resistant and clinical isolates of *S. aureus*, *S. epidermidis*. The following diameters of Ti NTs were used: 80, 120, 160 and 200 nm. The biofilm formation of tested strains on the specimens was evaluated using crystal violet staining (TCP method). It was found that smooth Ti had a lower antibiofilm effect compare to Ti NTs. However, tested samples with 160 nm and 200 nm diameters showed stronger antibiofilm activity. In the case of our research, pure Ti foil seems to have greater inhibition effect on biofilm formating bacteria compare to Ti NTs.

Further, in the research of Aydin and his team (Aydın *et al.*, 2018), they investigated TiO_2 NTs doped with silver synthesized via a two-step hydrothermal method. Bacterial strains of *S. aureus* and *E. coli* were involved in the examination of the antibacterial activity of silver-doped TiO_2 NTs. It was concluded that tested samples had similar antibacterial effects on all of the bacterial strains. As comparative substances, antibiotics (tetracycline and chloramphenicol) were used. In the case of *S. aureus* 3 and *S. aureus* 47, silver-doped TiO_2 NTs showed the most significant inhibition of bacterial growth comparable to tetracycline.

In the study of Kim *et al.*, the excellent antibacterial activity of Ti with the nanostructured surface was demonstrated (Kim *et al.*, 2018b). As well as other research papers, which are based on testing of nanostructured Ti for application mainly in medical fields, confirmed with their obtained results the promising application of Ti as an antimicrobial agent (Rosenbaum *et al.*, 2017; Cao *et al.*, 2018; Mahmoodian *et al.*, 2019).

4.3.3 The outcome of the research work

Nanostructured materials are fabricated to develop such biomaterials having tailored physical, chemical and biological properties. Simply, nanostructured biomaterials create an artificial microenvironment influencing cell adhesion, proliferation and differentiation together with antimicrobial activity. Among all metals, Ti is the material of choice clinically due to its mechanical strength and a relatively high degree of biocompatibility. The findings have contributed to knowledge about the behavior of both gram-positive and gram-negative biofilm-forming prokaryotic cells on TiO_2 NTs. Their growth was similar and the nanostructured surface did not influence the attachment of microbial cells. The well-defined nanostructured Ti surfaces within this study have provided new essential knowledge about its interaction with bacteria and eukaryotic cells which may be significant for developing novel nanostructured implantable devices.

This study was summarized into the manuscript which was subsequently submitted and published in the journal with impact factor as *Interaction of nanostructured TiO_2 biointerfaces with stern cells and biofilm-forming bacteria, International Journal of Polymeric Materials and Polymeric Biomaterials*, 2018, pp. 1-8. The article has even contributed to other research studies and it has been cited in seven articles dealing mainly with the improvement of the morphology of Ti NTs for significant application as medical implants.

4.4 Methods introduced to laboratory

4.4.1 DNA damage induced by conducting polymers

Damage of DNA is one of the critical issues when any redox-active materials are studied. There exists a variety of methods to study DNA damage. Within my study, the comet assay was introduced into the set of experimental methods in the biological laboratories of the Centre of Polymer Systems.

4.4.1.1 Comet assay

Comet assay seemed to be an interesting method appropriate for the main purpose of laboratory – the determination of interaction of a eukaryotic cell with materials. In this study, the DNA damage induced by extracts of PANI and PPy were tested by comet assay. This versatile, simple to perform and sensitive gel electrophoresis-based method allows the detection of DNA double-strand breaks, crosslinks, and base damage (Olive and Banath, 2006). The comet assay was originally developed for measure variation in DNA damage. Nowadays, applications of this method range from human and sentinel animal biomonitoring to measurement of DNA damage. The whole procedure is taken in detail from the protocol of Olive and Banath which is generally well-known and published (Olive and Banath, 2006).

Extracts of PANI and PPy powders, as well as CPs based colloids, were used for evaluation of their effect on DNA damage of tested mouse embryonic fibroblast cell (ATCC CRL-1658 NIH/3T3, USA). The testing was performed according to the protocol containing the precise procedure of comet assay as is described in chapter 3.3.3.1. The usable results were obtained in the case of extracts from CPs powders. Firstly, the PANI and PPy powders had to be extracted according to ISO 10993-12 in the ratio of 0.1 mg powder per 1 mL of cultivation medium. Extraction was performed in chemically inert closed containers using aseptic techniques at 37±1 °C under continuous stirring for 24±1 h. The parent extracts (100 %) were then diluted in a culture medium to obtain a series of dilutions with required concentrations. All extracts were used within 24 h. Cells were precultivated for 24 h. The culture medium was subsequently replaced with individual sample extracts. As a reference, cell cultivated in a pure medium was used. All tests were conducted in duplicates. Subsequently, the DNA damage of tested eukaryotic cells by prepared extracts was observed (Fig. 39). The obtained results showed that in the case of PANI-S and PANI-B the nuclei did not prove any damage, in comparison with the reference sample (pure gel without extract seeded by fibroblast cells). Whereas, PPy salt (PPy-S) and PPy base (PPy-B) had an even higher occurrence of DNA damage compared to sample with H_2O_2 (a reference compound).

When DNA is damaged, DNA double-strand bonds are broken. Because of these broken binding, the tail is created which is called a comet. Visually

determined comets of damaged nuclei caused by the action of PPy, both PPy-S and PPy-B, were larger than the comet of nucleus damaged by H_2O_2. Generally, this process is followed by a slide analysis using the fluorescence microscope. The taken image of the nuclei of the cells are subjected to measure of total intensity (DNA content) (Fig. 40), tail length, percent DNA in tail and tail moment. However, this step of the comet assay procedure was not set yet. The methodology is still improved.

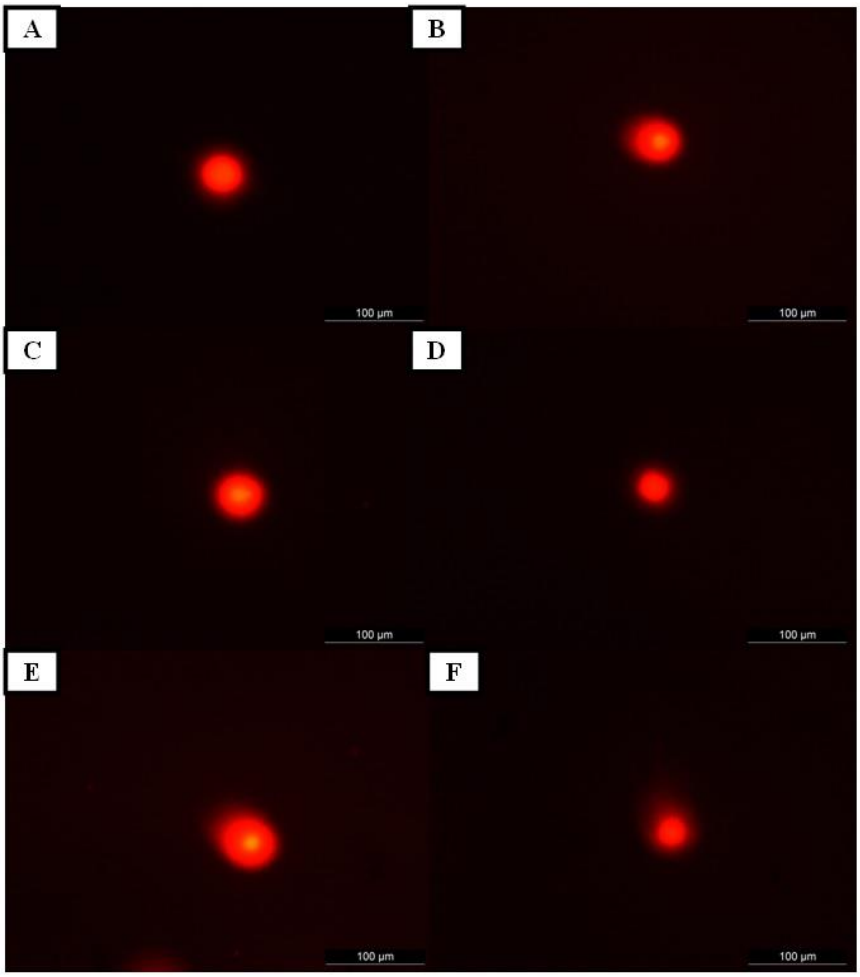


Fig. 39: Powders: A) Reference, B) H_2O_2, C) PANI-S, D) PANI-B, E) PPy-S, F) PPy-B. (The images were taken in the laboratory of the Centre of Polymer Systems by Nikola Mikušová).

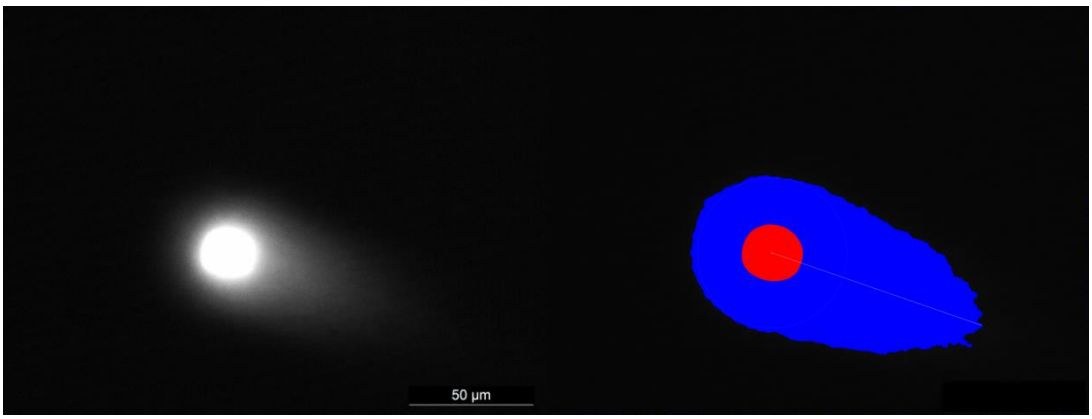


Fig. 40: The sample of image analysis (tail length analysis) – the cell nucleus damaged by the presence of H_2O_2. (The acknowledgment belongs to Assoc. Prof. Petr Ponížil)

One of the purposes of this doctoral work was the introduction of comet assay to the biological laboratory at the Center of Polymer Systems. This procedure is successfully added to the scope of the laboratory test.

4.4.1.2 Protein adsorption

Detection of protein adsorption was introduced in our biological laboratory through my Ph.D. study. The test was performed according to the protocol which was part of the Micro BCA Protein Assay kit. The colorimetric detection and quantitation of the total protein method were used for the evaluation of protein adsorption (using DMEM, BSA and serum) on the PANI-S and PANI-B surfaces. This unique, patented method uses bicinchoninic acid (BCA) as the detection reagent for Cu^{+1}. This atom is formed when Cu^{+2} is reduced by protein in an alkaline environment. A purple-colored reaction product is formed by the chelation of two molecules of BCA with one cuprous ion (Cu^{+1}). Thus, this water-soluble complex exhibits a strong absorbance at 562 nm that is linear with increasing protein concentrations. During protein adsorption measurement is possible to use two procedures, either test tube procedure or microplate procedure. The main approach does not vary, only the content of used chemicals is different. For this doctoral thesis, the microplate procedure was chosen, mainly due to using the small amount of chemicals and broad repeatability (taken from the instruction protocol included in Protein Assay kit).

5 SUMMARY OF WORK

Microorganisms attaching a material surface with subsequent biofilm formation are still a huge threat for biomedical and industrial applications. Moreover, microbial resistance against various antimicrobial compounds is higher compared to planktonic species. This fact forced an effort to find an appropriate modification of the surface of materials to provide intrinsic antimicrobial properties. The aim of this thesis is therefore related to this topic, concretely to reveal the antimicrobial properties of CPs and other materials in their native or modified form. To be more concrete, we wanted to reveal how the microbial attachment is connected with surface properties of materials and subsequently to modify these properties to minimalize biofouling.

As insufficient knowledge about the interaction between the CPs and biofilm-forming bacteria was found by the literature review at the beginning of my Ph.D. study. One of the tasks was to study the behavior of biofilm-forming species of bacteria on CP‘s surfaces. Within the practical part, the presented doctoral thesis is divided into four studies.

Firstly, biofilm formation on PANI films and thein surface properties were determined. Pure PANI surfaces and modified PANI surfaces with biological active acids were prepared. Those surfaces were subsequently subject to testing of antimicrobial effect using biofilm-forming bacteria and filamentous fungi. Briefly, PANI-S film did not show any inhibition of bacterial biofilm formation compared to the reference. In contrast, the PANI film doped with PAMPSA had a very significant antibacterial effect against all of the tested bacterial strains. This finding correlated with surface energy results where the lowest surface energy was measured in PANI-PAMPSA film. Concerning fungal biofilm formation, the target idea was to find if filamentous fungi are able to use compounds from tested surfaces as nutrients, for their growth and biofilm formation. The obtained knowledge about surface properties of CPs is of practical importance for various types of antimicrobial coatings, where PANI can be effectively applied both as an anti-corrosion agent and against pollution. Further, the study work at Jožef Stefan Institute in Ljubljana (Slovenia), was focused on the effect of oxygen plasma treatment on PANI surfaces. Plasma can change chemistry, morphology and other properties of a surface. Superhydrophilic surfaces prepared thanks to plasma attract water to create a thin layer which may consequently affect the bacterial attachment. Within this study the improved wettability of plasma-treated PANI films was confirmed, especially the significant observation was in PANI-B film. The roughness of the treated surfaces was decreased. It can positively influence biofilm formation of microorganisms which mostly prefer rougher substrate. Biological testing, unfortunately, did not prove the significant antimicrobial activity of plasma-treated PANI films and biocompatibility. Anyway, the obtained findings have

extended knowledge about surface properties of CPs after plasma treatment in contact with prokaryotic and eukaryotic cells.

The next study dealt with the evaluation of anticorrosive protection of metallic materials - protective coatings based on organic binders and corrosion-inhibiting pigments prepared in cooperation with Department of Paints and Organic Coatings, University of Pardubice. There was an effort to meet the ecological requirements as substitution of classical anticorrosive pigments by some other environmentally friendly pigments. The main goal was however to improve not only the anticorrosive activity but also the biological activity against bacterial and fungal strains.

The last research work studied TiO_2 NTs in contact with prokaryotic cells. This study was also performed in cooperation with Jožef Stefan Institute. From the experimental point of view, our contribution lies in the current promising nanomorphology of materials and in the comparison of the different sizes of NTs. TiO_2 NTs were uniformly distributed on the surface compared to pure Ti foil. One of the main contributions of this work is to understand more how Ti nanostructure and the size of NTs can influence bacterial behavior and attachment.

6 CONTRIBUTIONS TO SCIENCE AND PRACTICE

At the beginning of the doctoral study, there was insufficient information about the interaction between the biofilm-forming microorganisms and polymeric materials generally, and CPs especially. Presented work was therefore focused on the preparation, modification, and characterization of materials, predominantly composed of CPs. To fulfill the aim of the thesis, the studies focused on the modification of surface properties of CPs, incorporation of CPs into the polymeric coating, and on the impact of nanostructure prepared on TiO_2 surfaces were performed. An important novelty of all of these studies was the utilization of biofilm-forming species of bacteria and fungi. Newly acquired knowledge is of practical importance for various antimicrobial surface treatments.

The main contribution to the science of the reported work within the doctoral study course of TBU in Zlín, Technology of Macromolecular Compounds, can be found in the preparation and characterization of modified polymeric surfaces to increase their antimicrobial activity, together with maintaining or even improving biocompatibility. Prepared pure and modified PANI films were evaluated for biological and surface properties. The findings were subsequently summarized in the article published in *Chemical Papers* (Mikušová *et al.*, 2017). Further, the second article was focused on polymeric coatings with pigments prepared in cooperation with Department of Paints and Organic Coating, University of Pardubice. The obtained findings were published in *International Journal of Polymeric Materials and Polymeric Biomaterials* (Mikušová *et al.*, 2019). The presented thesis together with published articles brings novel approaches for modification of CPs and a deepening of knowledge about their surface properties in reaction with bacterial and fungal cells.

In addition to that, during the Ph.D. study, the third article based on TiO_2 NTs was published in *Materials Science and Engineering: C* (Kulkarni *et al.*, 2017; Mikušová as a co-author) in cooperation with Jožef Stefan Institute (Slovenia), Ljubljana. The composition of TiO_2 makes it resistant to corrosion and possessing high photocatalytic properties. Moreover, their application may be found in biomedicine (e.g. implants) due to hydrophilicity, good adhesion, antiseptic properties or non-toxicity. Currently, nanostructuring abounds with increasing popularity. The presence of nanostructure so improves adhesion and proliferation. Further, thanks to the active surface of this nanostructure, biologically active compounds can be absorbed on the surface of a material and subsequently increase the anti-biofouling properties.

The fourth article regarding adhesion, proliferation, and migration of NIH/3T3 cells on modified PANI surfaces was published *International Journal of Molecular Sciences* (Rejmontová *et al.*, 2016; Mikušová as a co-author).

Besides, during the study, new methodologies were introduced to the laboratories in the Centre of Polymer Systems at TBU in Zlín, concretely the

cultivation of various biofilm-forming bacteria and filamentous fungi, quantification of biofilm formation on the surfaces, determination of DNA damage using comet assay, and evaluation of protein adsorption on prepared polymeric surfaces.

Overall, the achieved outputs of applied research were already summarized in 4 articles and published in journals indexed in Web of Science. The results were also presented at 2 conferences (posters and contributions). To conclude, the field of Technology of macromolecular compounds has been thus enriched by new insights focused on the influence of material, especially CPs and their surface properties on microbial biofilm formation.

7 FUTURE PROSPECTIVE

In this Ph.D. thesis, we studied the antibiofilm effect of material related to the influence of microbial attachment using modified surface properties. This presented research has extended the knowledge about the interaction between pure and modified surfaces of CPs, and TiO_2 NTs with biofilm-forming bacteria, and mainly with filamentous fungi whose behavior has not been previously defined. This doctoral work may be further developed in several ways. Firstly, the micro and nanostructured surfaces may be prepared and subsequently, they can be functionalized using CPs to get material with unique biological properties. Further, the other possibility of how to extend biological testing of polymers is to use not only biofilm-forming strains but also the strains commonly occurring in a hospital environment. Moreover, within the biological testing of CPs, it would be beneficial to be focused on microorganisms occurring in an environment where they are applied, such as water or soil.

LIST OF REFERENCES

Abu-Thabit, N. Y. (2016) 'Chemical oxidative polymerization of polyaniline: A practical approach for preparation of smart conductive textiles', Journal of Chemical Education, 93(9), pp. 1606-1611.

Adhikari, A., Karpoormath, R., Radha, S., Singh, S. K., Mutthukannan, R., Bharate, G. and Vijayan, M. (2017) 'Corrosion resistant hydrophobic coating using modified conducting polyaniline', High Performance Polymers, 30(2), pp. 181-191.

Advincula, R. C. (2015) 'Conducting polymers with superhydrophobic effects as anticorrosion coating', Intelligent Coatings for Corrosion Control: Elsevier, pp. 409-430.

Al-Hussaini, A. S. and Eldars, W. (2014) 'Non-conventional synthesis and antibacterial activity of poly(aniline-co-o-phenylenediamine)/bentonite nanocomposites', Designed Monomers and Polymers, 17(5), pp. 458-465.

Amer, I., Mokrani, T., Jewell, L., Young, D. A. and Vosloo, H. C. M. (2015) 'Synthesis and characterization of sulfonated poly(p-phenylenediamine) prepared by different procedures', Polymer, 66, pp. 230-239.

Ansari, R. (2006) 'Polypyrrole conducting electroactive polymers: synthesis and stability studies', Journal of Chemistry, 3(4), pp. 186-201.

Anselme, K., Ploux, L. and Ponche, A. (2010) 'Cell/Material Interfaces: Influence of Surface Chemistry and Surface Topography on Cell Adhesion', Journal of Adhesion Science and Technology, 24(5), pp. 831-852.

Antizar-Ladislao, B. (2008) 'Environmental levels, toxicity and human exposure to tributyltin (TBT)-contaminated marine environment. A review', Environment International, 34(2), pp. 292-308.

Apetrei, R.-M., Carac, G., Bahrim, G., Ramanaviciene, A. and Ramanavicius, A. (2018) 'Modification of *Aspergillus niger* by conducting polymer, Polypyrrole, and the evaluation of electrochemical properties of modified cells', Bioelectrochemistry, 121, pp. 46-55.

Armelin, E., Oliver, R., Liesa, F., Iribarren, J. I., Estrany, F. and Alemán, C. (2007) 'Marine paint fomulations: Conducting polymers as anticorrosive additives', Progress in Organic Coatings, 59(1), pp. 46-52.

Armelin, E., Pla, R., Liesa, F., Ramis, X., Iribarren, J. I. and Aleman, C. (2008) 'Corrosion protection with polyaniline and polypyrrole as anticorrosive additives for epoxy paint', Corrosion Science, 50(3), pp. 721-728.

Arroyo, J., Akieh-Pirkanniemi, M., Lisak, G., Latonen, R.-M. and Bobacka, J. (2019) 'Electrochemically controlled transport of anions across polypyrrole-based membranes', Journal of membrane science, 581, pp. 50-57.

Atkinson, S., Chan, H., Neuendorf, A., Ng, S., Ong, T. and Young, D. J. (2000) 'Synthesis of the water-soluble, electrically conducting poly (5-aminonaphthalene-2-sulfonic acid)', Chemistry Letters, 29(3), pp. 276-277.

Au, K. M., Lu, Z., Matcher, S. J. and Armes, S. P. (2013) 'Anti-biofouling conducting polymer nanoparticles as a label-free optical contrast agent for high resolution subsurface biomedical imaging', Biomaterials, 34(35), pp. 8925-8940.

Ayad, M. M. and Zaki, E. A. (2008) 'Doping of polyaniline films with organic sulfonic acids in aqueous media and the effect of water on these doped films', European Polymer Journal, 44(11), pp. 3741-3747.

Aydın, M. T. A., Hoşgün, H. L., Dede, A. and Güven, K. (2018) 'Synthesis, characterization and antibacterial activity of silver-doped TiO_2 nanotubes', Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 205, pp. 503-507.

Bachmann, R. T. and Edyvean, R. G. J. (2006) 'Biofouling: an historic and contemporary review of its causes, consequences and control in drinking water distribution systems', Biofilms, 2(3), pp. 197-227.

Balint, R., Cassidy, N. J. and Cartmell, S. H. (2014) 'Conductive polymers: Towards a smart biomaterial for tissue engineering', Acta Biomaterialia, 10(6), pp. 2341-2353.

Banerjee, I., Pangule, R. C. and Kane, R. S. (2011) 'Antifouling Coatings: Recent Developments in the Design of Surfaces That Prevent Fouling by Proteins, Bacteria, and Marine Organisms', Advanced Materials, 23(6), pp. 690-718.

Bazaka, K., Jacob, M. V., Crawford, R. J. and Ivanova, E. P. (2011) 'Plasma-assisted surface modification of organic biopolymers to prevent bacterial attachment', Acta biomaterialia, 7(5), pp. 2015-2028.

Bhadra, J., Al-Thani, N. J., Madi, N. K. and Al-Maadeed, M. A. (2016) 'High performance sulfonic acid doped polyaniline–polystyrene blend ammonia gas sensors', Journal of Materials Science: Materials in Electronics, 27(8), pp. 8206-8216.

Binkauskiene, E., Lugauskas, A. and Bukauskas, V. (2013) 'The mycological effect on morphological, electrochemical and redox properties of the polyaniline surface', Surface and Interface Analysis, 45(11-12), pp. 1792-1798.

Blinova, N. V., Stejskal, J., Frechet, J. M. J. and Svec, F. (2012) 'Effect of reaction conditions on film morphology of polyaniline composite membranes for gas separation', Journal of Polymer Science Part a-Polymer Chemistry, 50(15), pp. 3077-3085.

Blinova, N. V., Stejskal, J., Trchová, M., Prokeš, J. and Omastová, M. (2007) 'Polyaniline and polypyrrole: A comparative study of the preparation', European Polymer Journal, 43(6), pp. 2331-2341.

Bober, P., Humpolicek, P., Pachernik, J., Stejskal, J. and Lindfors, T. (2015) 'Conducting polyaniline based cell culture substrate for embryonic stem cells and embryoid bodies', Rsc Advances, 5(62), pp. 50328-50335.

Boeva, Z. A. and Sergeyev, V. G. (2014) 'Polyaniline: Synthesis, properties, and application', Polymer Science Series C, 56(1), pp. 144-153.

Borthakur, P., Hussain, N., Darabdhara, G., Boruah, P. K., Sharma, B., Borthakur, P., Yadav, A. and Das, M. R. (2018) 'Adhesion of gram-negative bacteria onto α-Al_2O_3 nanoparticles: A study of surface behaviour and interaction mechanism', Journal of environmental chemical engineering, 6(4), pp. 3933-3941.

Bridier, A., Briandet, R., Thomas, V. and Dubois-Brissonnet, F. (2011) 'Resistance of bacterial biofilms to disinfectants: a review', Biofouling, 27(9), pp. 1017-1032.

Bruellhoff, K., Fiedler, J., Moller, M., Groll, J. and Brenner, R. E. (2010) 'Surface coating strategies to prevent biofilm formation on implant surfaces', Int J Artif Organs, 33(9), pp. 646-53.

Bryers, J. D. (2008) 'Medical Biofilms', Biotechnology and Bioengineering, 100(1), pp. 1-18.

Cabuk, M. and Gündüz, B. (2017) 'Controlling the optical properties of polyaniline doped by boric acid particles by changing their doping agent and initiator concentration', Applied Surface Science, 424, pp. 345-351.

Callister Jr, W. D. and Rethwisch, D. G. (2012) Fundamentals of materials science and engineering: an integrated approach. John Wiley & Sons.

Calvo, P. A., Rodriguez, J., Grande, H., Mecerreyes, D. and Pomposo, J. A. (2002) 'Chemical oxidative polymerization of pyrrole in the presence of m-hydroxybenzoic acid and m-hydroxycinnamic acid-related compounds', Synthetic Metals, 126(1), pp. 111-116.

Campoccia, D., Montanaro, L. and Arciola, C. R. (2013) 'A review of the biomaterials technologies for infection-resistant surfaces', Biomaterials, 34(34), pp. 8533-8554.

Cao, Y., Su, B., Chinnaraj, S., Jana, S., Bowen, L., Charlton, S., Duan, P., Jakubovics, N. S. and Chen, J. (2018) 'Nanostructured titanium surfaces exhibit recalcitrance towards Staphylococcus epidermidis biofilm formation', Scientific reports, 8(1), pp. 1071.

Chamovska, D., Porjazoska-Kujundziski, A. and Grchev, T. (2013) 'Polypyrrole coatings for corrosion protection of stainless steel', Zaštita materijala, 54(3), pp. 229-234.

Chauhan, N. P. S., Ameta, R., Ameta, R. and Ameta, S. C. (2010) 'Biological Activity of Emeraldine Bases of Polyaniline', Journal of The Indian Council of Chemists, 27(2), pp. 128-133.

Chelikani, R. (2007) Development of Novel Antibiofouling Materials from Natural Phenol Compounds. University of Toledo.

Chen, C., Qiu, S., Qin, S., Yan, G., Zhao, H. and Wang, L. (2017) 'Anticorrosion performance of epoxy coating containing reactive poly (o-phenylenediamine) nanoparticles', Int. J. Electrochem. Sci, 12, pp. 3417-3431.

Chouirfa, H., Bouloussa, H., Migonney, V. and Falentin-Daudré, C. (2019) 'Review of titanium surface modification techniques and coatings for antibacterial applications', Acta biomaterialia, 83, pp. 37-54.

Coad, B. R., Kidd, S. E., Ellis, D. H. and Griesser, H. J. (2014) 'Biomaterials surfaces capable of resisting fungal attachment and biofilm formation', Biotechnology advances, 32(2), pp. 296-307.

Collier, J. H., Camp, J. P., Hudson, T. W. and Schmidt, C. E. (2000) 'Synthesis and characterization of polypyrrole–hyaluronic acid composite biomaterials for tissue engineering applications', Journal of biomedical materials research, 50(4), pp. 574-584.

Cordeiro, E. R., Fernandes, A. W. C., Pereira, A. F. C., da Costa, M. M., Nascimento, M. L. F. and de Oliveira, H. P. (2015) 'Staphylococcus aureus Bifoilm Formation On Polypyrrole: An Electrical Overview', Quimica Nova, 38(8), pp. 1075-1079.

Costerton, J. W. (1995) 'Overview of Microbial Biofilms', Journal of Industrial Microbiology, 15(3), pp. 137-140.

Costerton, J. W. (1999) 'Introduction to biofilm', International Journal of Antimicrobial Agents, 11(3-4), pp. 217-221.

Costerton, W., Veeh, R., Shirtliff, M., Pasmore, M., Post, C. and Ehrlich, G. (2003) 'The application of biofilm science to the study and control of chronic bacterial infections', Journal of Clinical Investigation, 112(10), pp. 1466-1477.

da Silva Jr, F. A., Queiroz, J. C., Macedo, E. R., Fernandes, A. W., Freire, N. B., da Costa, M. M. and de Oliveira, H. P. (2016) 'Antibacterial behavior of polypyrrole: the influence of morphology and additives incorporation', Materials Science and Engineering: C, 62, pp. 317-322.

Dai, L. (2006) Intelligent Macromolecules for Smart Devices: From Materials Synthesis to Device Applications. Engineering Materials and Processes: Springer London.

De-la-Pinta, I., Cobos, M., Ibarretxe, J., Montoya, E., Eraso, E., Guraya, T. and Quindós, G. (2019) 'Effect of biomaterials hydrophobicity and roughness on biofilm development', Journal of Materials Science: Materials in Medicine, 30(7), pp. 77.

DeBerry, D. W. (1985) 'Modification of the electrochemical and corrosion behavior of stainless steels with an electroactive coating', Journal of the Electrochemical society, 132(5), pp. 1022-1026.

Dennstedt, M. and Zimmermann, J. (1887) 'Ueber die Einwirkung des Acetons auf das Pyrrol', Berichte der deutschen chemischen Gesellschaft, 20(2), pp. 2449-2459.

Deshpande, P. P. and Sazou, D. (2016) Corrosion protection of metals by intrinsically conducting polymers. CRC Press.

Deshpande, P. P., Vagge, S. T., Jagtap, S. P., Khairnar, R. S. and More, M. A. (2012) 'Conducting polyaniline based paints on low carbon steel for corrosion protection', Protection of Metals and Physical Chemistry of Surfaces, 48(3), pp. 356-360.

Diaz, A., Kanazawa, K. K. and Gardini, G. P. (1979) 'Electrochemical polymerization of pyrrole', Journal of the Chemical Society, Chemical Communications, (14), pp. 635-636.

do Nascimento, G. M., Sestrem, R. H. and Temperini, M. L. A. (2010) 'Structural characterization of poly-para-phenylenediamine–montmorillonite clay nanocomposites', Synthetic Metals, 160(23), pp. 2397-2403.

Domínguez-Aragón, A., Hernández-Escobar, C. A., Vega-Rios, A. and Zaragoza-Contreras, E. A. (2018) 'Poly (diphenylamine-co-aniline) copolymers for supercapacitor electrodes', Journal of Materials Science: Materials in Electronics, 29(18), pp. 15329-15338.

Dong, Y., Han, W. and Choi, H. (2018) 'Polyaniline Coated Core-Shell Typed Stimuli-Responsive Microspheres and Their Electrorheology', Polymers, 10(3), pp. 299.

Dunne, W. M. (2002) 'Bacterial adhesion: Seen any good biofilms lately?', Clinical Microbiology Reviews, 15(2), pp. 155-+.

Duran, B., Bereket, G. and Duran, M. (2012) 'Electrochemical synthesis and characterization of poly (m-phenylenediamine) films on copper for corrosion protection', Progress in Organic Coatings, 73(2-3), pp. 162-168.

Ehrenstein, G. W. (2001) Polymeric Materials: Structure, Properties, Applications. Hanser Publishers.

ElNahrawy, A. M., Haroun, A. A., Hamadneh, I. and Al-Dujaili, A. H. (2017) 'Conducting cellulose/TiO_2 composites by in situ polymerization of pyrrole', Carbohydrate polymers, 168, pp. 182-190.

Fateixa, S., Neves, M. C., Almeida, A., Oliveira, J. and Trindade, T. (2009) 'Anti-fungal activity of SiO_2/Ag_2S nanocomposites against *Aspergillus niger*', Colloids and Surfaces B: Biointerfaces, 74(1), pp. 304-308.

Fedorko, P., Trznadel, M., Pron, A., Djurado, D., Planès, J. and Travers, J. (2010) 'New analytical approach to the insulator–metal transition in conductive polyaniline', Synthetic Metals, 160(15-16), pp. 1668-1671.

Felgueiras, H., Antunes, J., Martins, M. and Barbosa, M. (2018) 'Fundamentals of protein and cell interactions in biomaterials', Peptides and Proteins as Biomaterials for Tissue Regeneration and Repair: Elsevier, pp. 1-27.

Flemming, H.-C. (2002) 'Biofouling in water systems–cases, causes and countermeasures', Applied microbiology and biotechnology, 59(6), pp. 629-640.

Flemming, H. C. and Wingender, J. (2001) 'Relevance of microbial extracellular polymeric substances (EPSs)--Part I: Structural and ecological aspects', Water Sci Technol, 43(6), pp. 1-8.

Flemming, H. C. and Wingender, J. (2010) 'The biofilm matrix', Nature Reviews Microbiology, 8(9), pp. 623-633.

Flemming, H. C., Wingender, J., Szewzyk, U., Steinberg, P., Rice, S. A. and Kjelleberg, S. (2016) 'Biofilms: an emergent form of bacterial life', Nature Reviews Microbiology, 14(9), pp. 563-575.

Francis, L. and Roberts, C. 2016. Chapter 6-Dispersion and Solution Processes. Materials Processing. Boston: Academic Press.

Fried, J. R. (2014) Polymer science and technology. Pearson Education.

Fullenkamp, D. E., Rivera, J. G., Gong, Y.-k., Lau, K. A., He, L., Varshney, R. and Messersmith, P. B. (2012) 'Mussel-inspired silver-releasing antibacterial hydrogels', Biomaterials, 33(15), pp. 3783-3791.

Fusetani, N. (2004) 'Biofouling and antifouling', Natural product reports, 21(1), pp. 94-104.

Gallarato, L. A., Mulko, L. E., Dardanelli, M. S., Barbero, C. A., Acevedo, D. F. and Yslas, E. I. (2017) 'Synergistic effect of polyaniline coverage and surface microstructure on the inhibition of Pseudomonas aeruginosa biofilm formation', Colloids and Surfaces B: Biointerfaces, 150, pp. 1-7.

Garai, A. and Nandi, A. K. (2009) 'Tuning of different polyaniline nanostructures from a coacervate gel/sol template', Synthetic Metals, 159(7-8), pp. 757-760.

Garcia-Cabezon, C., Garcia-Hernandez, C., Rodriguez-Mendez, M. and Martin-Pedrosa, F. (2020) 'A new strategy for corrosion protection of porous stainless steel using polypyrrole films', Journal of Materials Science & Technology, 37, pp. 85-95.

Garrett, T. R., Bhakoo, M. and Zhang, Z. (2008) 'Bacterial adhesion and biofilms on surfaces', Progress in Natural Science, 18(9), pp. 1049-1056.

Ghasemi-Mobarakeh, L., Prabhakaran, M. P., Morshed, M., Nasr-Esfahani, M. H., Baharvand, H., Kiani, S., Al-Deyab, S. and Ramakrishna, S. (2011) 'Application of conductive polymers, scaffolds and electrical stimulation for nerve tissue engineering', Journal of Tissue Engineering and Regenerative Medicine, 5(4), pp. E17-E35.

Gilbert, P. and Moore, L. (2005) 'Cationic antiseptics: diversity of action under a common epithet', Journal of applied microbiology, 99(4), pp. 703-715.

Gizdavic-Nikolaidis, M. R., Bennett, J., Zujovic, Z., Swift, S. and Bowmaker, G. A. (2012) 'Characterization and antimicrobial efficacy of acetone extracted aniline oligomers', Synthetic Metals, 162(13-14), pp. 1114-1119.

Gizdavic-Nikolaidis, M. R., Bennett, J. R., Swift, S., Easteal, A. J. and Ambrose, M. (2011) 'Broad spectrum antimicrobial activity of functionalized polyanilines', Acta Biomaterialia, 7(12), pp. 4204-4209.

Gomez, H., Ram, M. K., Alvi, F., Villalba, P., Stefanakos, E. and Kumar, A. (2011) 'Graphene-conducting polymer nanocomposite as novel electrode for supercapacitors', Journal of Power Sources, 196(8), pp. 4102-4108.

Gong, K., Guo, S., Zhao, Y., Hu, Q., Liu, H., Sun, D., Li, M., Qiu, B. and Guo, Z. (2018) 'Bacteria cell templated porous polyaniline facilitated detoxification and recovery of hexavalent chromium', Journal of Materials Chemistry A, 6(35), pp. 16824-16832.

Goto, H., Yoneyama, H., Togashi, F., Ohta, R., Tsujimoto, A., Kita, E. and Ohshima, K.-i. (2008) 'Preparation of conducting polymers by electrochemical methods and demonstration of a polymer battery', Journal of Chemical Education, 85(8), pp. 1067.

Gottesman, R., Shukla, S., Perkas, N., Solovyov, L. A., Nitzan, Y. and Gedanken, A. (2011) 'Sonochemical Coating of Paper by Microbiocidal Silver Nanoparticles', Langmuir, 27(2), pp. 720-726.

Grari, O., Taouil, A. E., Dhouibi, L., Buron, C. and Lallemand, F. (2015) 'Multilayered polypyrrole–SiO_2 composite coatings for functionalization of stainless steel: characterization and corrosion protection behavior', Progress in Organic Coatings, 88, pp. 48-53.

Green, R. A., Hassarati, R. T., Goding, J. A., Baek, S., Lovell, N. H., Martens, P. J. and Poole-Warren, L. A. (2012) 'Conductive hydrogels: mechanically robust hybrids for use as biomaterials', Macromolecular bioscience, 12(4), pp. 494-501.

Gribkova, O. L., Nekrasov, A. A., Trchova, M., Ivanov, V. F., Sazikov, V. I., Razova, A. B., Tverskoy, V. A. and Vannikov, A. V. (2011) 'Chemical synthesis of polyaniline in the presence of poly(amidosulfonic acids) with different rigidity of the polymer chain', Polymer, 52(12), pp. 2474-2484.

Guimard, N. K., Gomez, N. and Schmidt, C. E. (2007) 'Conducting polymers in biomedical engineering', Progress in polymer science, 32(8-9), pp. 876-921.
Guo, B. and Ma, P. X. (2018) 'Conducting polymers for tissue engineering', Biomacromolecules, 19(6), pp. 1764-1782.

Guo, Z. G., Liu, W. M. and Su, B. L. (2011) 'Superhydrophobic surfaces: From natural to biomimetic to functional', Journal of Colloid and Interface Science, 353(2), pp. 335-355.

Gvozdenovic, M. M., Jugovic, B. Z., Stevanovic, J. S. and Grgur, B. N. (2014) 'Electrochemical synthesis of electroconducting polymers', Hemijska Industrija, 68(6), pp. 673-684.

Hall-Stoodley, L., Costerton, J. W. and Stoodley, P. (2004) 'Bacterial biofilms: From the natural environment to infectious diseases', Nature Reviews Microbiology, 2(2), pp. 95-108.

Hasan, J., Crawford, R. J. and Ivanova, E. P. (2013) 'Antibacterial surfaces: the quest for a new generation of biomaterials', Trends in biotechnology, 31(5), pp. 295-304.

Hashim, A. A. (2010) Polymer Thin films. InTech 2010, p. 336.

Hegemann, D., Brunner, H. and Oehr, C. (2003) 'Plasma treatment of polymers for surface and adhesion improvement', Nuclear instruments and methods in physics research section B: Beam interactions with materials and atoms, 208, pp. 281-286.

Hong, S. Y. and Marynick, D. S. (1992) 'Understanding the conformational stability and electronic structures of modified polymers based on polythiophene', Macromolecules, 25(18), pp. 4652-4657.

Hu, H., Siu, V. S., Gifford, S. M., Kim, S., Lu, M., Meyer, P. and Stolovitzky, G. A. (2017) 'Bio-inspired silicon nanospikes fabricated by metal-assisted chemical etching for antibacterial surfaces', Applied Physics Letters, 111(25), pp. 253701.

Huang, Y., Li, H., Wang, Z., Zhu, M., Pei, Z., Xue, Q., Huang, Y. and Zhi, C. (2016) 'Nanostructured polypyrrole as a flexible electrode material of supercapacitor', Nano Energy, 22, pp. 422-438.

Hui, N., Sun, X., Niu, S. and Luo, X. (2017) 'PEGylated polyaniline nanofibers: antifouling and conducting biomaterial for electrochemical DNA sensing', ACS applied materials & interfaces, 9(3), pp. 2914-2923.

Humpolicek, P., Kucekova, Z., Kasparkova, V., Pelkova, J., Modic, M., Junkar, I., Trchova, M., Bober, P., Stejskal, J. and Lehocky, M. (2015) 'Blood coagulation and platelet adhesion on polyaniline films', Colloids and Surfaces B-Biointerfaces, 133, pp. 278-285.

Ibanez, J. G., Rincón, M. E., Gutierrez-Granados, S., Chahma, M. h., Jaramillo-Quintero, O. A. and Frontana-Uribe, B. A. (2018) 'Conducting polymers in the fields of energy, environmental remediation, and chemical–chiral sensors', Chemical reviews, 118(9), pp. 4731-4816.

Inzelt, G. (2008) Conducting Polymers: A New Era in Electrochemistry. Monographs in electroanalytical chemistry and electrochemistry: Springer.

Istakova, O. I., Konev, D. V., Glazkov, A. T., Medvedeva, T. O., Zolotukhina, E. V. and Vorotyntsev, M. A. (2019) 'Electrochemical synthesis of polypyrrole in powder form', Journal of Solid State Electrochemistry, 23(1), pp. 251-258.

Ivanova, E. P., Hasan, J., Webb, H. K., Truong, V. K., Watson, G. S., Watson, J. A., Baulin, V. A., Pogodin, S., Wang, J. Y. and Tobin, M. J. (2012) 'Natural bactericidal surfaces: mechanical rupture of Pseudomonas aeruginosa cells by cicada wings', Small, 8(16), pp. 2489-2494.

Jaggessar, A., Shahali, H., Mathew, A. and Yarlagadda, P. K. (2017) 'Bio-mimicking nano and micro-structured surface fabrication for antibacterial properties in medical implants', Journal of nanobiotechnology, 15(1), pp. 64.

Jaidev and Ramaprabhu, S. (2012) 'Poly(p-phenylenediamine)/graphene nanocomposites for supercapacitor applications', Journal of Materials Chemistry, 22(36), pp. 18775-18783.

Jain, A., Duvvuri, L. S., Farah, S., Beyth, N., Domb, A. J. and Khan, W. (2014) 'Antimicrobial Polymers', Advanced Healthcare Materials, 3(12), pp. 1969-1985.

Jamal, M., Tasneem, U., Hussain, T. and Andleeb, S. (2015) Bacterial Biofilm: Its Composition, Formation and Role in Human Infections.

Jaymand, M. (2013) 'Recent progress in chemical modification of polyaniline', Progress in Polymer Science, 38(9), pp. 1287-1306.

Jian-xun, D., Key Laboratory of Polymer, E. and Key Laboratory of Polymer Ecomaterials. (2015) 'Synergism of electroactive polymeric materials and electrical stimulation promotes peripheral nerve repair', pp. 1781-1782.

Jindai, K., Nakade, K., Sagawa, T., Kojima, H., Shimizu, T., Shingubara, S. and Ito, T. (2019) 'Investigation of nanostructure-based bactericidal effect derived from a cicada wing by using QCM-D', Materials Today: Proceedings, 7, pp. 492-496.

Jucker, B. A., Harms, H. and Zehnder, A. (1996) 'Adhesion of the positively charged bacterium Stenotrophomonas (Xanthomonas) maltophilia 70401 to glass and Teflon', Journal of bacteriology, 178(18), pp. 5472-5479.

Junkar, I., Vesel, A., Cvelbar, U., Mozetič, M. and Strnad, S. (2009) 'Influence of oxygen and nitrogen plasma treatment on polyethylene terephthalate (PET) polymers', Vacuum, 84(1), pp. 83-85.

Kadam, A. V. and Patil, S. B. (2018) 'Polyaniline globules as a catalyst for WO3 nanoparticles for supercapacitor application', Materials Research Express, 5(8), pp. 085036.

Kalendová, A. (2002) 'Comparison of the efficiencies of anticorrosive pigments based on phosphates', Pigment & Resin Technology, 31(6), pp. 381-388.

Kalendová, A., Sapurina, I., Stejskal, J. and Veselý, D. (2008) 'Anticorrosion properties of polyaniline-coated pigments in organic coatings', Corrosion Science, 50(12), pp. 3549-3560.

Kalendová, A., Veselý, D., Kohl, M. and Stejskal, J. (2014) 'Effect of surface treatment of pigment particles with polypyrrole and polyaniline phosphate on their corrosion inhibiting properties in organic coatings', Progress in Organic Coatings, 77(9), pp. 1465-1483.

Kalendová, A., Veselý, D., Kohl, M. and Stejskal, J. (2015) 'Anticorrosion efficiency of zinc-filled epoxy coatings containing conducting polymers and pigments', Progress in Organic Coatings, 78, pp. 1-20.

Kaloni, T. P., Giesbrecht, P. K., Schreckenbach, G. and Freund, M. S. (2017) 'Polythiophene: From fundamental perspectives to applications', Chemistry of Materials, 29(24), pp. 10248-10283.

Kanaparthy, A. and Kanaparthy, R. (2012) 'Biofilms-The Unforgiving Film in Dentistry (Clinical Endodontic Biofilms)', Dentistry, 2(145), pp. 2161-1122.1000145.

Kanazawa, K. K., Diaz, A., Gill, W., Grant, P., Street, G., Gardini, G. P. and Kwak, J. (1980) 'Polypyrrole: an electrochemically synthesized conducting organic polymer', Synthetic Metals, 1(3), pp. 329-336.

Kang, E., Neoh, K. and Tan, K. (1998) 'Polyaniline: a polymer with many interesting intrinsic redox states', Progress in Polymer Science, 23(2), pp. 277-324.

Kaplan, D. L. (1998) 'Introduction to biopolymers from renewable resources', Biopolymers from renewable resources: Springer, pp. 1-29.

Karlsson, J., Ytreberg, E. and Eklund, B. (2010) 'Toxicity of anti-fouling paints for use on ships and leisure boats to non-target organisms representing three trophic levels', Environmental pollution, 158(3), pp. 681-687.

Kashyap, G., Meghwal, K., Ameta, C., Ameta, R. and Punjabi, P. B. (2019) 'Synthesis of ascorbic acid-doped oligoaniline, its drug composites and study of their antibacterial behavior', Polymer Bulletin, 76(5), pp. 2439-2451.

Kaur, G., Adhikari, R., Cass, P., Bown, M. and Gunatillake, P. (2015) 'Electrically conductive polymers and composites for biomedical applications', Rsc Advances, 5(47), pp. 37553-37567.

Kausaite-Minkstimiene, A., Mazeiko, V., Ramanaviciene, A. and Ramanavicius, A. (2015) 'Evaluation of chemical synthesis of polypyrrole particles', Colloids and Surfaces A: Physicochemical and Engineering Aspects, 483, pp. 224-231.

Kaynak, A. and Foitzik, R. C. (2010) 'Synthesis and Characterization of Soluble Conducting Polymers', Research Journal of Textile and Apparel, 14(2), pp. 45-52.

Keevil, C. and Walker, J. (1992) Nomarski DIC microscopy and image analysis.
Khan, F., Manivasagan, P., Pham, D. T. N., Oh, J., Kim, S.-K. and Kim, Y.-M. (2019) 'Antibiofilm and antivirulence properties of chitosan-polypyrrole nanocomposites to Pseudomonas aeruginosa', Microbial pathogenesis, 128, pp. 363-373.

Khanh, T. T., Lee, R.-J., Kilmartin, P. A., Khan, M. A., Khorram, M. S., Tamm, T. and Kiefer, R. (2018) 'Actuation increase in polypyrrole bilayer by photo-activated dopants', Synthetic Metals, 246, pp. 57-63.

Kim, D. W., Moon, J.-M., Park, S., Choi, J. S. and Cho, W. K. (2018a) 'Facile and effective antibacterial coatings on various oxide substrates', Journal of Industrial and Engineering Chemistry, 68, pp. 42-47.

Kim, S., Park, C., Cheon, K.-H., Jung, H.-D., Song, J., Kim, H.-E. and Jang, T.-S. (2018b) 'Antibacterial and bioactive properties of stabilized silver on titanium with a nanostructured surface for dental applications', Applied Surface Science, 451, pp. 232-240.

Kishi, R., Hiroki, K., Tominaga, T., Sano, K. I., Okuzaki, H., Martinez, J. G., Otero, T. F. and Osada, Y. (2012) 'Electro-conductive double-network hydrogels', Journal of Polymer Science Part B: Polymer Physics, 50(11), pp. 790-796.

Kochkodan, V. and Hilal, N. (2015) 'A comprehensive review on surface modified polymer membranes for biofouling mitigation', Desalination, 356, pp. 187-207.

Kohl, M. and Kalendová, A. (2015) 'Anticorrosion properties of organic coatings containing polyphenylenediamine phosphate', Advances in Science and Technology Research Journal, 9(28), pp. 47--50.

Koutny, M., Sancelme, M., Dabin, C., Pichon, N., Delort, A. M. and Lemaire, J. (2006) 'Acquired biodegradability of polyethylenes containing pro-oxidant additives', Polymer Degradation and Stability, 91(7), pp. 1495-1503.

Kucekova, Z., Kasparkova, V., Humpolicek, P., Sevcikova, P. and Stejskal, J. (2013) 'Antibacterial properties of polyaniline-silver films', Chemical Papers, 67(8), pp. 1103-1108.

Kucekova, Z., Rejmontova, P., Humpolicek, P., Kasparkova, V., Bober, P., Saha, P. and Stejskal, J. (2017) 'Cytotoxicity of poly(p-phenylenediamine)', Chemical Papers, 71(2), pp. 367-372.

Kulkarni, M., Junkar, I., Humpolíček, P., Capáková, Z., Radaszkiewicz, K. A., Mikušová, N., Pacherník, J., Lehocký, M., Iglič, A. and Hanáčková, M. (2017) 'Interaction of nanostructured TiO_2 biointerfaces with stem cells and biofilm-forming bacteria', Materials Science and Engineering: C, 77, pp. 500-507.

Kulkarni, M., Mazare, A., Park, J., Gongadze, E., Killian, M. S., Kralj, S., von der Mark, K., Iglič, A. and Schmuki, P. (2016) 'Protein interactions with layers of TiO_2 nanotube and nanopore arrays: Morphology and surface charge influence', Acta Biomaterialia, 45, pp. 357-366.

Kumar, A., Jangir, L. K., Kumari, Y., Kumar, M., Kumar, V. and Awasthi, K. (2016) 'Electrical behavior of dual-morphology polyaniline', Journal of Applied Polymer Science, 133(41).

Kumar, C. G. and Anand, S. (1998) 'Significance of microbial biofilms in food industry: a review', International journal of food microbiology, 42(1-2), pp. 9-27.

Kumar, R., Singh, S. and Yadav, B. (2015) 'Conducting Polymers: Synthesis, Properties and Applications', International Advanced Research Journal in Science, Engineering and Technology, 2(11), pp. 110-124.

Kumar, R. and Yadav, B. (2016) 'Humidity sensing investigation on nanostructured polyaniline synthesized via chemical polymerization method', Materials Letters, 167, pp. 300-302.

Kundu, K. and Giri, D. (1996) 'Evolution of the electronic structure of cyclic polythiophene upon bipolaron doping', The Journal of chemical physics, 105(24), pp. 11075-11080.

Lai, J., Yi, Y., Zhu, P., Shen, J., Wu, K., Zhang, L. and Liu, J. (2016) 'Polyaniline-based glucose biosensor: A review', Journal of Electroanalytical Chemistry, 782, pp. 138-153.

Le, T.-H., Kim, Y. and Yoon, H. (2017) 'Electrical and Electrochemical Properties of Conducting Polymers', Polymers, 9(4), pp. 150.

Li, G. F., Martinez, C., Janata, J., Smith, J. A., Josowicz, M. and Semancik, S. (2004) 'Effect of morphology on the response of polyaniline-based conductometric gas sensors: Nanofibers vs. thin films', Electrochemical and Solid State Letters, 7(10), pp. H44-H47.

Li, K., Zhang, H., Tang, T., Tang, Y., Wang, Y. and Jia, J. (2016) 'Facile electrochemical polymerization of polypyrrole film applied as cathode material in dual rotating disk photo fuel cell', Journal of Power Sources, 324, pp. 368-377.

Li, P., Zhang, D., Xu, Y., Ni, C., Shi, G., Sang, X. and Cong, H. (2018) 'Hierarchical porous polyaniline supercapacitor electrode from polyaniline/silica self- aggregates', Polymer International, 67(12), pp. 1670-1676.

Li, Y. H. and Tian, X. L. (2012) 'Quorum Sensing and Bacterial Social Interactions in Biofilms', Sensors, 12(3), pp. 2519-2538.

Liang, J. Z., Li, B. and Ruan, J. Q. (2015) 'Crystallization properties and thermal stability of polypropylene composites filled with wollastonite', Polymer Testing, 42, pp. 185-191.

Lin, W.-t., Zhang, Y.-y., Tan, H.-l., Ao, H.-y., Duan, Z.-l., He, G. and Tang, T.-t. (2016) 'Inhibited Bacterial Adhesion and Biofilm Formation on Quaternized Chitosan-Loaded Titania Nanotubes with Various Diameters', Materials, 9(3), pp. 155.

Liu, C., Geng, L., Yu, Y., Zhang, Y., Zhao, B., Zhang, S. and Zhao, Q. (2018) 'Reduction of bacterial adhesion on Ag-TiO2 coatings', Materials Letters, 218, pp. 334-336.

Liu, K. and Jiang, L. (2011) 'Bio-inspired design of multiscale structures for function integration', Nano Today, 6(2), pp. 155-175.

Lugauskas, A. (2003) 'Micromycetes as deterioration agents of polymeric materials', International Biodeterioration & Biodegradation, 52(4), pp. 233-242.

Luo, S.-C. (2013) 'Conducting Polymers as Biointerfaces and Biomaterials: A Perspective for a Special Issue of Polymer Reviews', Polymer Reviews, 53(3), pp. 303-310.

MacDiarmid, A. G. and Epstein, A. J. (1995) 'Secondary doping in polyaniline', Synthetic Metals, 69(1), pp. 85-92.

Macdiarmid, A. G., Mammone, R. J., Kaner, R. B. and Porter, S. J. (1985) 'The Concept of Doping of Conducting Polymers - The Role of Reduction Potentials', Philosophical Transactions of the Royal Society a-Mathematical Physical and Engineering Sciences, 314(1528), pp. 3-15.

Mah, T. F. and O'Toole, G. A. (2001) 'Mechanisms of biofilm resistance to antimicrobial agents', Trends Microbiol, 9(1), pp. 34-9.

Mahmoodian, M., Hajihoseini, H., Mohajerzadeh, S. and Fathipour, M. (2019) 'Nano patterning and fabrication of single polypyrrole nanowires by electron beam lithography', Synthetic Metals, 249, pp. 14-24.

Mandić, Z., Duić, L. and Kovačiček, F. (1997) 'The influence of counter-ions on nucleation and growth of electrochemically synthesized polyaniline film', Electrochimica Acta, 42(9), pp. 1389-1402.

Manna, J., Goswami, S., Shilpa, N., Sahu, N. and Rana, R. K. (2015) 'Biomimetic Method To Assemble Nanostructured Ag@ZnO on Cotton Fabrics: Application as Self-Cleaning Flexible Materials with Visible-Light Photocatalysis and Antibacterial Activities', ACS Applied Materials & Interfaces, 7(15), pp. 8076-8082.

Mara, D. and Horan, N. J. (2003) Handbook of Water and Wastewater Microbiology. Elsevier Science.

Marshall, K. C. (1986) 'Adsorption and adhesion processes in microbial growth at interfaces', Advances in Colloid and Interface Science, 25, pp. 59-86.

Mayer, C., Moritz, R., Kirschner, C., Borchard, W., Maibaum, R., Wingender, J. and Flemming, H. C. (1999) 'The role of intermolecular interactions: studies on model systems for bacterial biofilms', International Journal of Biological Macromolecules, 26(1), pp. 3-16.

McFarland, J. and L'Engle, E. M. (1907) 'The Specific Nature of Opsonins', Journal of the American Medical Association, XLIX(14), pp. 1178-1181.

Mengoli, G., Munari, M. T., Bianco, P. and Musiani, M. M. (1981) 'Anodic synthesis of polyaniline coatings onto fe sheets', Journal of Applied Polymer Science, 26(12), pp. 4247-4257.

Miah, M., Iqbal, Z. and Lai, E. P. C. (2012) 'Rapid CE-UV evaluation of polypyrrole-coated magnetic nanoparticles for selective binding of endocrine disrupting compounds and pharmaceuticals by aromatic interactions', Analytical Methods, 4(9), pp. 2866-2878.

Mikušová, N., Humpolíček, P., Růžička, J., Capáková, Z., Janů, K., Kašpárková, V., Bober, P., Stejskal, J., Koutný, M., Filatová, K., Lehocký, M. and Ponížil, P. (2017) 'Formation of bacterial and fungal biofilm on conducting polyaniline', Chemical Papers, 71(2), pp. 505-512.

Mikušová, N., Nechvilová, K., Kalendová, A., Hájková, T., Capáková, Z., Junkar, I., Lehocký, M., Mozetič, M. and Humpolíček, P. (2019) 'The effect of composition of a polymeric coating on the biofilm formation of bacteria and filamentous fungi', International Journal of Polymeric Materials and Polymeric Biomaterials, 68(4), pp. 152-159.

Mleziva, J. and Šňupárek, J. Polymery: výroba, struktura, vlastnosti a použití, 2. vydání. Praha, Sobottáles, 2000. lo.

Modaresifar, K., Azizian, S., Ganjian, M., Fratila-Apachitei, L. E. and Zadpoor, A. A. (2019) 'Bactericidal effects of nanopatterns: A systematic review', Acta Biomaterialia, 83, pp. 29-36.

Montag, D., Frant, M., Horn, H. and Liefeith, K. (2012) 'Dependence of the initial adhesion of biofilm forming Pseudomonas putida mt2 on physico-chemical material properties', Biofouling, 28(3), pp. 315-327.

Montemor, M. F. (2014) 'Functional and smart coatings for corrosion protection: A review of recent advances', Surface and Coatings Technology, 258, pp. 17-37.

Moyen, E., Hama, A., Ismailova, E., Assaud, L., Malliaras, G., Hanbücken, M. and Owens, R. M. (2016) 'Nanostructured conducting polymers for stiffness controlled cell adhesion', Nanotechnology, 27(7), pp. 074001.

Muchová, M., Růžička, J., Julinová, M., Doležalová, M., Houser, J., Koutný, M. and Buňková, L. (2009) 'Xanthan and gellan degradation by bacteria of activated sludge', Water Science and Technology, 60(4), pp. 965-973.

Murr, L. E. (2015) 'Biomimetics and Biologically Inspired Materials', in Murr, L.E. (ed.) Handbook of Materials Structures, Properties, Processing and Performance. Cham: Springer International Publishing, pp. 521-552.

Muthusankar, E., Vignesh Kumar, S., Rajagopalan, N. and Ragupathy, D. (2018) 'Synthesis and Characterization of Co-Polymer Nanocomposite Film and its Enhanced Antimicrobial Behavior', BioNanoScience, 8(4), pp. 1008-1013.

Müller, B. and Fischer, S. (2006) 'Epoxy ester resins as corrosion inhibitors for aluminium and zinc pigments', Corrosion Science, 48(9), pp. 2406-2416.
Nambiar, S. and Yeow, J. T. W. (2011) 'Conductive polymer-based sensors for biomedical applications', Biosensors & Bioelectronics, 26(5), pp. 1825-1832.

Nanlin Shi, X. G. U. O. H. J. J. G. C. S. U. N. K. Y. (2006) 'Antibacterial Effect of the Conducting Polyaniline', Journal of Materials Sciences and Technology, 22(03), pp. 289-290.

Narendrakumar, K., Kulkarni, M., Addison, O., Mazare, A., Junkar, I., Schmuki, P., Sammons, R. and Iglič, A. (2015) 'Adherence of oral streptococci to nanostructured titanium surfaces', Dental Materials, 31(12), pp. 1460-1468.

Naseri, M. G., Saion, E. B., Hashim, M., Shaari, A. H. and Ahangar, H. A. (2011) 'Synthesis and characterization of zinc ferrite nanoparticles by a thermal treatment method', Solid State Communications, 151(14), pp. 1031-1035.

Nautiyal, A., Qiao, M., Cook, J. E., Zhang, X. and Huang, T.-S. (2018) 'High performance polypyrrole coating for corrosion protection and biocidal applications', Applied Surface Science, 427, pp. 922-930.

Netala, V. R., Kotakadi, V. S., Nagam, V., Bobbu, P., Ghosh, S. B. and Tartte, V. (2015) 'First report of biomimetic synthesis of silver nanoparticles using aqueous callus extract of Centella asiatica and their antimicrobial activity', Applied Nanoscience, 5(7), pp. 801-807.

Ning, C., Zhou, Z., Tan, G., Zhu, Y. and Mao, C. (2018) 'Electroactive polymers for tissue regeneration: Developments and perspectives', Progress in Polymer Science, 81, pp. 144-162.

Oil and Association, C. C. (1993) 'Surface coatings–raw materials and their usage', Chapman&Hall, London.

Olive, P. L. and Banath, J. P. (2006) 'The comet assay: a method to measure DNA damage in individual cells', Nature Protocols, 1(1), pp. 23-29.

Omastova, M., Bober, P., Moravkova, Z., Perinka, N., Kaplanova, M., Syrovy, T., Hromadkova, J., Trchova, M. and Stejskal, J. (2014) 'Towards conducting inks: Polypyrrole-silver colloids', Electrochimica Acta, 122, pp. 296-302.

Oudghiri-Hassani, H. (2015) 'Synthesis, characterization and catalytic performance of iron molybdate $Fe_2(MoO_4)_3$ nanoparticles', Catalysis Communications, 60, pp. 19-22.

Oyharcabal, M., Olinga, T., Foulc, M. P., Lacomme, S., Gontier, E. and Vigneras, V. (2013) 'Influence of the morphology of polyaniline on the microwave absorption properties of epoxy polyaniline composites', Composites Science and Technology, 74, pp. 107-112.

Pak, J.-J., Bahgat, M. and Paek, M.-K. (2009) 'Synthesis of nanocrystalline Fe–W composite through hydrogen reduction of thermally synthesized iron tungstate, Fe_2WO_6', Journal of Alloys and Compounds, 477(1), pp. 357-363.

Pan, T. (2013) 'Intrinsically Conducting Polymer-Based Heavy-Duty and Environmentally Friendly Coating System for Corrosion Protection of Structural Steels', Spectroscopy Letters, 46(4), pp. 268-276.

Parakhonskiy, B. and Shchukin, D. (2015) 'Polypyrrole Microcontainers: Electrochemical Synthesis and Characterization', Langmuir, 31(33), pp. 9214-9218.

Parashar, U. K., Kumar, V., Bera, T., Saxena, P. S., Nath, G., Srivastava, S. K., Giri, R. and Srivastava, A. (2011) 'Study of mechanism of enhanced antibacterial activity by green synthesis of silver nanoparticles', Nanotechnology, 22(41), pp. 415104.

Pavithra, D. and Doble, M. (2008) 'Biofilm formation, bacterial adhesion and host response on polymeric implants—issues and prevention', Biomedical Materials, 3(3), pp. 034003.

Pious, C. and Thomas, S. (2016) 'Polymeric Materials—Structure, Properties, and Applications', Printing on Polymers: Fundamentals and Applications, 1st ed.; Izdebska, J., Thomas, S., Eds, pp. 21-40.

Pogodin, S., Hasan, J., Baulin, Vladimir A., Webb, Hayden K., Truong, Vi K., Phong Nguyen, The H., Boshkovikj, V., Fluke, Christopher J., Watson, Gregory S., Watson, Jolanta A., Crawford, Russell J. and Ivanova, Elena P. (2013) 'Biophysical Model of Bacterial Cell Interactions with Nanopatterned Cicada Wing Surfaces', Biophysical Journal, 104(4), pp. 835-840.

Prokeš, J., Stejskal, J. and Omastová, M. 2001. Polyanilin a polypyrrol: dva představitelé vodivých polymerů. Chemické Listy.

Prokeš, P. and Kalendová, A. (2007) 'Anticorrosion efficiency of coatings containing metallic pigments', Journal of Physics and Chemistry of Solids, 68(5), pp. 1083-1086.

Pyo, M. and Hwang, J.-H. (2009) 'Conductivity changes of dodecylbezensulfonic acid-doped polyaniline during pressure loading/unloading', Synthetic Metals, 159(7), pp. 700-704.

Queffélec, C., Petit, M., Janvier, P., Knight, D. A. and Bujoli, B. (2012) 'Surface Modification Using Phosphonic Acids and Esters', Chemical Reviews, 112(7), pp. 3777-3807.

Rabin, N., Zheng, Y., Opoku-Temeng, C., Du, Y. X., Bonsu, E. and Sintim, H. O. (2015) 'Biofilm formation mechanisms and targets for developing antibiofilm agents', Future Medicinal Chemistry, 7(4), pp. 493-512.

Rao, P. R. (2003) 'Biomimetics', Sadhana, 28(3), pp. 657-676.

Rasmussen, S. C. (2011) 'Electrically Conducting Plastics: Revising the History of Conjugated Organic Polymers', 100+ Years of Plastics. Leo Baekeland and Beyond: Vol. 1080 ACS Symposium Series: American Chemical Society, pp. 147-163.

Ravikumar, T., Murata, H., Koepsel, R. R. and Russell, A. J. (2006) 'Surface-Active Antifungal Polyquaternary Amine', Biomacromolecules, 7(10), pp. 2762-2769.

Rehim, M. H. A., Ismail, N., Badawy, A. and Turky, G. (2011) 'Poly phenylenediamine and its TiO_2 composite as hydrogen storage material', Materials Chemistry and Physics, 128(3), pp. 507-513.

Rejmontová, P., Capáková, Z., Mikušová, N., Maráková, N., Kašpárková, V., Lehocký, M. and Humpolíček, P. (2016) 'Adhesion, Proliferation and Migration of NIH/3T3 Cells on Modified Polyaniline Surfaces', International Journal of Molecular Sciences, 17(9).

Renner, L. D. and Weibel, D. B. (2011) 'Physicochemical regulation of biofilm formation', Mrs Bulletin, 36(5), pp. 347-355.

Rikhari, B., Pugal Mani, S. and Rajendran, N. (2018) 'Electrochemical behavior of polypyrrole/chitosan composite coating on Ti metal for biomedical applications', Carbohydrate Polymers, 189, pp. 126-137.

Rodney, M. D. (2002) 'Biofilms: Microbial Life on Surfaces', Emerging Infectious Disease journal, 8(9), pp. 881.

Rodríguez-Hernández, J. (2016) Polymers against Microorganisms: On the Race to Efficient Antimicrobial Materials. Springer International Publishing.

Rohwerder, M. (2009) 'Conducting polymers for corrosion protection: a review', International Journal of Materials Research, 100(10), pp. 1331-1342.

Rosenbaum, J., Versace, D. L., Abbad-Andallousi, S., Pires, R., Azevedo, C., Cénédese, P. and Dubot, P. (2017) 'Antibacterial properties of nanostructured Cu–TiO_2 surfaces for dental implants', Biomaterials Science, 5(3), pp. 455-462.

Rummel, C. D., Jahnke, A., Gorokhova, E., Kühnel, D. and Schmitt-Jansen, M. (2017) 'Impacts of Biofilm Formation on the Fate and Potential Effects of Microplastic in the Aquatic Environment', Environmental Science & Technology Letters, 4(7), pp. 258-267.

Rutherford, S. T. and Bassler, B. L. (2012) 'Bacterial Quorum Sensing: Its Role in Virulence and Possibilities for Its Control', Cold Spring Harbor Perspectives in Medicine, 2(11).

Sambhy, V., Peterson, B. R. and Sen, A. (2008) 'Multifunctional Silane Polymers for Persistent Surface Derivatization and Their Antimicrobial Properties', Langmuir, 24(14), pp. 7549-7558.

Sambyal, P., Ruhi, G., Bhandari, H. and Dhawan, S. K. (2015) 'Advanced anti corrosive properties of poly(aniline-co-o-toluidine)/flyash composite coatings', Surface and Coatings Technology, 272, pp. 129-140.

Sapurina, I., Riede, A. and Stejskal, J. (2001) 'In-situ polymerized polyaniline films: 3. Film formation', Synthetic Metals, 123(3), pp. 503-507.

Sarikaya, M., Tamerler, C., Jen, A. K. Y., Schulten, K. and Baneyx, F. (2003) 'Molecular biomimetics: nanotechnology through biology', Nature Materials, 2(9), pp. 577-585.

Sathiyanarayanan, S., Muthukrishnan, S., Venkatachari, G. and Trivedi, D. C. (2005) 'Corrosion protection of steel by polyaniline (PANI) pigmented paint coating', Progress in Organic Coatings, 53(4), pp. 297-301.

Satpathy, S., Sen, S. K., Pattanaik, S. and Raut, S. (2016) 'Review on bacterial biofilm: An universal cause of contamination', Biocatalysis and Agricultural Biotechnology, 7, pp. 56-66.

Scharifker, B. R., García-Pastoriza, E. and Marino, W. (1991) 'The growth of polypyrrole films on electrodes', Journal of Electroanalytical Chemistry and Interfacial Electrochemistry, 300(1), pp. 85-98.

Shah, A. S., Firlak, M., Berrow, R. S., Halcovitch, R. N., Baldock, J. S., Yousafzai, M. B., Hathout, M. R. and Hardy, G. J. (2018) 'Electrochemically Enhanced Drug Delivery Using Polypyrrole Films', Materials, 11(7).

Sharma, R., Sims, R. A. and Mazumder, M. K. (2002) 'Modification of Surface Properties of Polymeric Materials', Journal of Arkansas Acamedy of Science, 56, pp. 157-161.

Shi, S., Zhang, Z. and Yu, L. (2017) 'Hydrophobic polyaniline/modified SiO2 coatings for anticorrosion protection', Synthetic Metals, 233, pp. 94-100.

Shirakawa, H., Louis, E. J., MacDiarmid, A. G., Chiang, C. K. and Heeger, A. J. (1977) 'Synthesis of electrically conducting organic polymers: halogen derivatives of polyacetylene, (CH)', Journal of the Chemical Society, Chemical Communications, (16), pp. 578-580.

Sim, B. and Choi, H. J. (2015) 'Facile synthesis of polyaniline nanotubes and their enhanced stimuli-response under electric fields', RSC Advances, 5(16), pp. 11905-11912.

Sitaram, S. P., Stoffer, J. O. and O'Keefe, T. J. (1997) 'Application of conducting polymers in corrosion protection', Journal of Coatings Technology, 69(866), pp. 65-69.

Skotheim, T. (1986) Handbook of Conducting Polymers [Vol 1-2].

Sladek, R. E. J., Stoffels, E., Walraven, R., Tielbeek, P. J. A. and Koolhoven, R. A. (2004) 'Plasma treatment of dental cavities: a feasibility study', IEEE Transactions on Plasma Science, 32(4), pp. 1540-1543.

Slepička, P., Trostová, S., Slepičková Kasálková, N., Kolská, Z., Sajdl, P. and Švorčík, V. (2012) 'Surface Modification of Biopolymers by Argon Plasma and Thermal Treatment', Plasma Processes and Polymers, 9(2), pp. 197-206.

Sobolewski, P., Piwowarczyk, M. and Fray, M. E. (2016) 'Polymer–Graphene Nanocomposite Materials for Electrochemical Biosensing', Macromolecular Bioscience, 16(7), pp. 944-957.

Sokolova, M. P., Smirnov, M. A., Kasatkin, I. A., Dmitriev, I. Y., Saprykina, N. N., Toikka, A. M., Lahderanta, E. and Elyashevich, G. K. (2017) 'Interaction of Polyaniline with Surface of Carbon Steel', International Journal of Polymer Science, 2017, pp. 9.

Song, F., Koo, H. and Ren, D. (2015) 'Effects of Material Properties on Bacterial Adhesion and Biofilm Formation', Journal of Dental Research, 94(8), pp. 1027-1034.

Spinks, G. M., Dominis, A. J., Wallace, G. G. and Tallman, D. E. (2002) 'Electroactive conducting polymers for corrosion control', Journal of Solid State Electrochemistry, 6(2), pp. 85-100.

Stejskal, J. 'Polyanilin: Vodivý polymer', Praktické kurzy z fyziky a chemie: Otevřená věda.

Stejskal, J. 2013. Conducting polymer-silver composites. Chemical Papers.

Stejskal, J. (2015) 'Polymers of phenylenediamines', Progress in Polymer Science, 41, pp. 1-31.

Stejskal, J. and Gilbert, R. G. 2002. Polyaniline. Preparation of a conducting polymer(IUPAC Technical Report). Pure and Applied Chemistry.

Stejskal, J., Prokes, J. and Trchova, M. (2008) 'Reprotonation of polyaniline: A route to various conducting polymer materials', Reactive & Functional Polymers, 68(9), pp. 1355-1361.

Stejskal, J., Prokeš, J. and Trchová, M. (2008) 'Reprotonation of polyaniline: A route to various conducting polymer materials', Reactive and Functional Polymers, 68(9), pp. 1355-1361.

Stejskal, J., Prokeš, J. and Trchová, M. (2014) 'Reprotonated polyanilines: The stability of conductivity at elevated temperature', Polymer Degradation and Stability, 102, pp. 67-73.

Stejskal, J. and Sapurina, I. (2005) 'Polyaniline: Thin films and colloidal dispersions - (IUPAC technical report)', Pure and Applied Chemistry, 77(5), pp. 815-826.

Stejskal, J., Sapurina, I., Prokeš, J. and Zemek, J. (1999) 'In-situ polymerized polyaniline films', Synthetic Metals, 105(3), pp. 195-202.

Stejskal, J., Trchová, M., Bober, P., Humpolíček, P., Kašpárková, V., Sapurina, I., Shishov, M. A. and Varga, M. 'Conducting Polymers: Polyaniline'.

Stejskal, J., Trchová, M., Bober, P., Humpolíček, P., Kašpárková, V., Sapurina, I., Shishov, M. A. and Varga, M. (2015) 'Conducting Polymers: Polyaniline', Encyclopedia of Polymer Science and Technology, pp. 1-44.

Stoodley, P., Boyle, J. D., Dodds, I. and Lappin-Scott, H. M. (1997) 'Consensus model of biofilm structure'.

Strahl, H. and Hamoen, L. W. (2010) 'Membrane potential is important for bacterial cell division', Proceedings of the National Academy of Sciences, 107(27), pp. 12281.

Szuwarzynski, M., Wolski, K. and Zapotoczny, S. (2016) 'Enhanced stability of conductive polyacetylene in ladder-like surface-grafted brushes', Polymer Chemistry, 7(36), pp. 5664-5670.

Tan, Y. and Ghandi, K. (2013) 'Kinetics and mechanism of pyrrole chemical polymerization', Synthetic Metals, 175, pp. 183-191.

Teughels, W., Van Assche, N., Sliepen, I. and Quirynen, M. (2006) 'Effect of material characteristics and/or surface topography on biofilm development', Clinical Oral Implants Research, 17(S2), pp. 68-81.

Thomas, C., Zong, K., Schottland, P. and Reynolds, J. (2000) 'Poly (3, 4-alkylenedioxypyrrole) s as highly stable aqueous-compatible conducting polymers with biomedical implications', Advanced Materials, 12(3), pp. 222-225.

Tiller, J. C., Liao, C.-J., Lewis, K. and Klibanov, A. M. (2001) 'Designing surfaces that kill bacteria on contact', Proceedings of the National Academy of Sciences, 98(11), pp. 5981.

Tolker-Nielsen, T. and Molin, S. (2000) 'Spatial Organization of Microbial Biofilm Communities', Microb Ecol, 40(2), pp. 75-84.

Tomšík, E., Morávková, Z., Stejskal, J., Trchová, M. and Zemek, J. (2012) 'In situ polymerized polyaniline films: The top and the bottom', Synthetic Metals, 162(24), pp. 2401-2405.

Tourillon, G. (1986) 'Polythiophene and its derivatives', Handbook of conducting polymers, 1, pp. 293.

Toyofuku, M., Inaba, T., Kiyokawa, T., Obana, N., Yawata, Y. and Nomura, N. (2016) 'Environmental factors that shape biofilm formation', Bioscience, Biotechnology, and Biochemistry, 80(1), pp. 7-12.

Trojan, M., Brandová, D. and Šolc, Z. (1987) 'Study of the thermal preparation and stability of tetrametaphosphates of bivalent metals', Thermochimica Acta, 110(Supplement C), pp. 343-358.

Ul Ahad, I., Bartnik, A., Fiedorowicz, H., Kostecki, J., Korczyc, B., Ciach, T. and Brabazon, D. (2014) 'Surface modification of polymers for biocompatibility via exposure to extreme ultraviolet radiation', Journal of Biomedical Materials Research Part A, 102(9), pp. 3298-3310.

Ungureanu, C., Popescu, S., Purcel, G., Tofan, V., Popescu, M., Salageanu, A. and Pirvu, C. (2014) 'Improved antibacterial behavior of titanium surface with torularhodin-polypyrrole film', Materials Science & Engineering C-Materials for Biological Applications, 42, pp. 726-733.

Varesano, A., Vineis, C., Aluigi, A., Rombaldoni, F., Tonetti, C. and Mazzuchetti, G. (2013) 'Antibacterial Efficacy of Polypyrrole in Textile Applications', Fibers and Polymers, 14(1), pp. 36-42.

Varesano, A., Vineis, C., Tonetti, C., Mazzuchetti, G. and Bobba, V. (2015) 'Antibacterial property on Gram-positive bacteria of polypyrrole-coated fabrics', Journal of Applied Polymer Science, 132(12).

Veerasubramani, G. K., Krishnamoorthy, K., Radhakrishnan, S., Kim, N.-J. and Kim, S. J. (2016) 'In-situ chemical oxidative polymerization of aniline monomer in the presence of cobalt molybdate for supercapacitor applications', Journal of Industrial and Engineering Chemistry, 36, pp. 163-168.

Vernitskaya, T. y. V. and Efimov, O. N. (1997) 'Polypyrrole: a conducting polymer; its synthesis, properties and applications', Russian Chemical Reviews, 66(5), pp. 443-457.

Veselý, D., Kalendová, A. and Němec, P. (2010) 'Properties of organic coatings depending on chemical composition and structure of pigment particles', Surface and Coatings Technology, 204(12), pp. 2032-2037.

Visai, L., De Nardo, L., Punta, C., Melone, L., Cigada, A., Imbriani, M. and Arciola, C. R. (2011) 'Titanium Oxide Antibacterial Surfaces in Biomedical Devices', The International Journal of Artificial Organs, 34(9), pp. 929-946.

Vroman, L. (1962) 'Effect of Adsorbed Proteins on the Wettability of Hydrophilic and Hydrophobic Solids', Nature, 196(4853), pp. 476-477.

Wanieck, K., Fayemi, P.-E., Maranzana, N., Zollfrank, C. and Jacobs, S. (2017) 'Biomimetics and its tools'.

Watnick, P. and Kolter, R. (2000) 'Biofilm, city of microbes', J Bacteriol, 182(10), pp. 2675-9.

Wei, Q. and Ma, Z. L. (2013) 'Biofilm Matrix and Its Regulation in Pseudomonas aeruginosa', International Journal of Molecular Sciences, 14(10).

Wen, T.-C., Huang, L.-M. and Gopalan, A. (2001) 'An in situ spectroelectrochemical investigation of the copolymerization of diaminobenzenesulfonic acid with aniline and its derivatives', Electrochimica Acta, 46(16), pp. 2463-2475.

Wu, W., Giese, R. F., Jr. and van Oss, C. J. (1995) 'Evaluation of the Lifshitz-van der Waals/Acid-Base Approach To Determine Surface Tension Components', Langmuir, 11(1), pp. 379-382.

Yao, C.-J., Xie, J., Wu, Z., Xu, Z. J., Zhang, S. and Zhang, Q. (2019) 'A Conjugated Copolymer of N-Phenyl-p-phenylenediamine and Pyrene as Promising Cathode for Rechargeable Lithium–Ion Batteries', Chemistry – An Asian Journal, 14(13), pp. 2210-2214.

Yehgambaram, P., Prasad, R. G. S. V., Jakka, V. S., Aparna, R. S. L. and Phani, A. R. (2013) 'Antifungal activity of nanostructured polyaniline combined with fluconazole', Journal of Pharmacy Research, 6(1), pp. 26-31.

Yuan, W., Han, G., Xiao, Y., Chang, Y., Liu, C., Li, M., Li, Y. and Zhang, Y. (2016) 'Flexible electrochemical capacitors based on polypyrrole/carbon fibers via chemical polymerization of pyrrole vapor', Applied Surface Science, 377, pp. 274-282.

Zaitsev, A., Lacoste, A., Poncin-Epaillard, F., Bès, A. and Debarnot, D. (2017) 'Nanotexturing of plasma-polymer thin films using argon plasma treatment', Surface and Coatings Technology, 330, pp. 196-203.

Zengin, H., Aksin, G., Zengin, G., Kahraman, M. and Kilic, I. H. (2019) 'Preparation and Characterization of Conductive Polyaniline/Silver Nanocomposite Films and Their Antimicrobial Studies', Polymer Engineering & Science, 59(S1), pp. E182-E194.

Zhang, Q., Dong, H. and Hu, W. (2018) 'Electrochemical polymerization for two-dimensional conjugated polymers', Journal of Materials Chemistry C, 6(40), pp. 10672-10686.

Zhou, H. H., Chen, H., Luo, S. L., Lu, G. W., Wei, W. Z. and Kuang, Y. F. (2005) 'The effect of the polyaniline morphology on the performance of polyaniline supercapacitors', Journal of Solid State Electrochemistry, 9(8), pp. 574-580.

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LIST OF SYMBOLS AND ABBREVIATIONS

AFM	Atomic force microscopy
AG	Afterglow regime
BSA	Bovine serum albumine
BSC	Bovine calf serum
CCM	Czech Collection of Microorganisms
CP/CPs	Conducting polymer/Conducting polymers
CSA	Camphorsulfonic acid
DI	Deionized water
DMEM	Dulbecco’s minimum essential medium
EG	Ethylene glycol
EMP	Electroactive polymeric material
EPS	Extracellular polymeric substance
HF	Hydrofluoric acid
MEBB	Melt-extract bouillon broth
NP/NPs	Nanoparticle/nanoparticles
NT/NTs	Nanotube/Nanotubes
PA	Polyacetylene
PAMPSA	Poly(2-acrylamido-2-methyl-1-propanesulfonic acid)
PANI	Polyaniline
PANI-B	Polyaniline base
PANI-S	Polyaniline salt
PBS	Phosphate buffered saline

PEDOT	Poly(3,4-ethylendioxythiophene)
PP	Polypropylene
PPy	Polypyrrole
PPDA	Poly(phenylenediamine)
PPP	Poly(p-phenylene)
PPV	Poly(phenylenevinylene)
PPy-S	Polypyrrole salt
PPy-B	Polypyrrole base
PS	Polystyrene
PTA	Phosphotungstic acid
PTh	Polythiophene
Ra	Average surface roughness
SEE system	Surface energy evaluation system
SEM	Scanning electron microscopy
SDS	Sodium dodecyl sulfate
SIMS	Secondary ion mass spectrometry
VCP	Volume concentration of pigment
WR	Working reagent
WCA	Water contact angle
XPS	X-ray photoelectron spectroscopy
γ_{tot}	Total surface energy
γ_{LW}	Disperse component
γ_{AB}	Acid-base (polar) component

8 LIST OF PUBLICATIONS

During the doctoral study, the results and new findings were published in four articles in journals with impact factors (two as the first author, two as co-author). The list of published articles is given below.

Author's publication activities

Articles published in journals indexed in Web of Science:

Mikušová, N., Humpolíček, P., Růžička, J., Capáková, Z. *et al.* Formation of bacterial and fungal biofilm on conducting polyaniline. *Chemical Papers*, 2017, *71(2)*, pp. 505-512. DOI: 10.1007/s11696-016-0073-8. ISSN 2585-7290.

Mikušová, N., Nechvilová, K., Kalendová, A., Hájková, T., Capáková, Z., Humpolíček, P. *et al*. The effect of composition of a polymeric coating on the biofilm formation of bacteria and filamentous fungi. *International Journal of Polymeric Materials and Polymeric Biomaterials*, 2019, *68(4)*, pp. 152-159. DOI: 10.1080/00914037.2018.1429435. ISSN 0091-4037.

Kulkarni, M., Junkar, I., Humpolíček, P., **Mikušová, N.** Interaction of nanostructured TiO_2 biointerfaces with stem cells and biofilm-forming bacteria. *Materials Science and Engineering: C*, 2017, *77*, pp. 500-507. DOI: 10.1016/j.msec.2017.03.174. ISSN 09284931.

Rejmontová, P., Capáková, Z., **Mikušová, N.**, Maráková, N., Kašpárková, V., Lehocký, Humpolíček, P. Adhesion, Proliferation and Migration of NIH/3T3 Cells on Modified Polyaniline Surfaces. *International Journal of Molecular Sciences*, 2016, *17(9)*, pp. 1439. DOI: 10.3390/ijms17091439. ISSN 1422-0067.

CONFERENCE CONTRIBUTIONS

The conference proceedings:

Mikušová, N., Koutný, M., Kuceková, Z., Humpolíček, P., Rejmontová, P. The Biofilm Formation on Conductive Polymer Polyaniline.
VII. mezinárodní konference Bioimplantologie, Brno, 2015, Czech Republic.

Mikušová, N. The Influence of Surface Properties of Materials on Biofilm Formation. ISSN 1948-5948. DOI: 10.4172/1948-5948-C1-035.
4th World Congress and Expo on Applied Microbiology, 2017, Madrid, Spain.

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The influence of surface properties of materials on biofilm formation

Vliv povrchových vlastností materiálů na tvorbu biofilmu

Doctoral Thesis