
Antimicrobial Modifications of Polymers

Vladimir Sedlarik

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/56222>

1. Introduction

Polymers have been used for decades instead of metal, glass and wood in many applications due to their superior physicochemical properties, in addition to others, as well as for reasons of economy. For example, commercially available thermoplastic polymers, such as polyolefins, are hydrophobic and biologically inert. This has made them indispensable in the packaging industry, distribution of food stuffs and other perishable commodities [1]. Another example is agriculture, where plastics have largely replaced glass in the construction of green houses, in addition to which they have gained a unique position in the growing of soft fruit and vegetables over mulching films [2]. Increasing demands on polymer materials have led to their further development. In some applications, the polymer products also possess, besides the passive function (e.g. packaging or structural) an active function (e.g. protective and/or indicative). The polymeric materials with resistance to microbial colonization and pathogenic microorganism spreading (antimicrobial polymers) have been one of the examples of the active material functionality. The antimicrobial polymers are expected to protect against negative impact of the pathogenic microorganisms, which can seriously affect the society from the viewpoint of both health damages and unwanted economical loads connected with that.

This chapter is focused on antimicrobial modifications of polymer materials intended for medical devices production. Firstly, a brief introduction into the field of medical application of polymers is presented. Considering the fact that polymer medical devices are often connected with occurrence of nosocomial infections, the next part refers to this phenomenon and its causes. One of the possibilities of reducing of the infection occurrence is aimed at polymer modification. It is a key topic of the third part of the chapter. Finally, the methodology of the polymer materials antimicrobial properties determination is shown, together with references to the relevant standards.

2. Polymers in medicine

The opening paragraph highlights that polymers are now part and parcel of everyday life - in form of frequently utilized items (disposable packaging, textile fibers, and construction materials) as well as in specialized and complex fields such as electronics and pharmaceuticals. Polymers have found applications in medicine, too. They are commonly used to produce various medical devices, including implants, drug carriers, protective packaging materials, and healthcare items [3]. Such applications of polymers in the medicinal sphere are shown in Table 1.

Polymer	Medical device applications
Polyethylene	Orthopedic implants, containers, catheters, non-woven textiles
Polypropylene	Disposable items (e.g. syringes), non-woven textiles, membranes, sutures
Polyurethanes	Films, tubing, catheters
Polyvinylchloride	Catheters, tubing
Polyamides	Sutures, packaging, dental implants
Polyethylene terephthalate	Sutures, artificial vascular grafts
Polycarbonates	Containers, construction material
Poly(methyl methacrylate)	Membranes, implants, part of bone cement
Polydimethylsiloxane	Implants, catheters
Polytetrafluoroethylene	Artificial vascular grafts, catheters (albeit rarely)
Polyether ether ketone	Tubing
Poly lactide	Resorbable implants

Table 1. Examples of synthetic polymer applications in medicine [4]

As can be seen in Table 1, polymer-based medical devices can consist of both synthetic non-biodegradable and biodegradable polymers, which are referred to as *bio-resorbable* in this instance. Another division of polymer biomaterials (i.e. materials used to produce medical devices) can be delineated according to polymer origin, i.e. synthetic and natural (e.g. cellulose, collagen, and derivatives) [5].

One might consider polymer an ideal biomaterial due to the benefits mentioned above. However, the use of polymers in medicine has a significant disadvantage. Polymer-based surfaces are often vulnerable to bacterial attack, which can give rise to serious complications in the form of so-called Nosocomial infections (see further sections) [6, 7]. Therefore, developing new polymer systems exhibiting antimicrobial activity has been the focus of extensive research, with numerous methods being applied to prevent and control the occurrence of these complications.

3. Bacterial colonization of polymer surfaces and its relation to nosocomial infections

Nosocomial infections have affected human beings for ages. There is evidence of surgical operations even taking place in ancient Egypt. However, the mortality rate of patients remained high due to subsequent complications. This was normal until the 1860s, when Dr. Joseph Lister introduced the practice of aseptic surgery, enabling surgeons to perform a greater variety of complex operations. Figure 1 shows a time scale of milestones in medical device development.

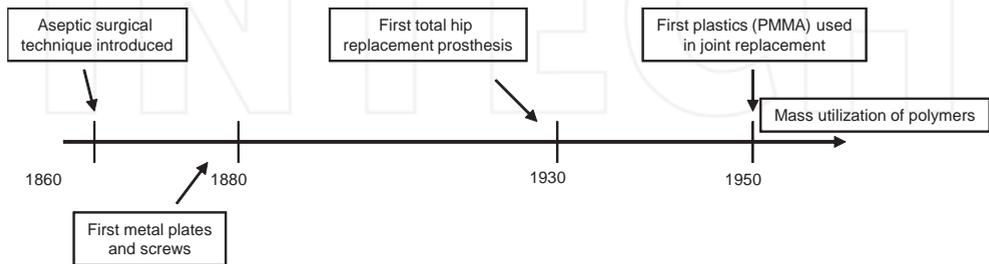


Figure 1. Notable milestones in relation to medical devices

Nosocomial infections (from Greek *nosos* = disease, *comeo* = care) are a consequence of bacteria colonizing the surface of a polymer-based medical device (see Figure 2).

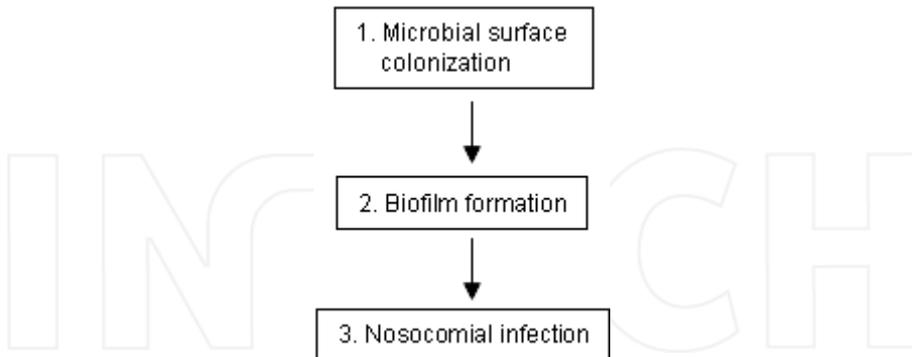


Figure 2. Consequences of microbial surface adhesion

A nosocomial infection (also known as secondary infections, hospital acquired infections) can be defined as a health complication that arises in the therapy of a patient in a medical facility. The infection can be both endogenous (e.g. mediated by surgery treatment) and exogenous (spread via an external environment) [8].

The repercussions of nosocomial infections are clear. Statistics reveal that the average incidence of secondary infections affects 8% of all hospitalized patients (for Great Britain it is 10%, Italy 6.7%, and Finland 8.7%). The United States observes the occurrence of nosocomial infection in over 1.7 million cases per year. Such health complications cause over 90,000 deaths a year. It is estimated that the annual cost of treatment of nosocomial infections brought about by mediated complications is between 4.5 and 11 billion US dollars [8, 9]. Nosocomial infections are often in the form of urinary infections (40%), wound infections (25%) and nosocomial pneumonia (20%).

The vast majority of the above-mentioned health complications are linked with the usage of medical devices. As already indicated in Figure 2, nosocomial infections are related to microbial colonisation and subsequent biofilm formation on the surfaces of polymer medical devices. Following text refers to the fundamentals of these phenomena.

3.1. Bacterial adhesion

Investigating the primary cause of bacterial adhesion on surfaces has proven a challenge to microbiologists for several decades. Initial works were, however, focused on microbial surface colonization in an aqueous environment. *Marshall* described the process of reversible and irreversible adhesion of the bacterial strain *Pseudomonas sp.* on a glass substrate in 1985 [10]. Further studies showed that the adhesion process can take from several seconds to a few minutes [11]. Extensive attention was paid to researching the extent of bacterial adhesion and colonization versus the physicochemical properties of surfaces. Here it is worth mentioning the example of polyethylene. The polyolefin is considered susceptible to bacterial colonization by various bacterial strains in a marine water environment, this being the cause of polymer deterioration [12]. However, a rise in unexplained infections in medical facilities brought this area to the attention of scientists. Reference is made later to various theories that exist to explain the primary cause of bacterial surface adhesion (for instance, thermodynamic theory [13]). Nevertheless, none of them has led to full clarification of this phenomenon [14].

One of the highest regarded approaches in bacteria adhesion process description was DVLO theory (Derjaguin, Landau, Verwey and Overbeek theory), which considers individual bacteria as colloid particles. These particles interact with surfaces on the basis of their charges [15]. Originally DVLO theory was introduced to explain stabilities in colloid systems in the 1940's. It formulates the stability of colloid systems as a measure of potential energy consisting of two components: (i) interactions of attraction based on Van der Waals forces; (ii) electrostatic repulsive forces. However, DVLO theory does not take into consideration a number of facts, and it cannot be accepted as satisfactory. Firstly, bacteria have an inhomogeneous surface. The surface of bacteria can be covered by an extracellular polysaccharide or peptide. Furthermore, appendages that protrude from the body of bacteria might be present (e.g. flagella) and significantly affect bacteria-surface interactions [16]. Conducting an experimentally confirmed process for irreversible adhesion also raises questions regarding the applicability of DVLO theory [10].

Generally, it is well accepted that bacterial adhesion on abiotic surfaces is mediated by non-specific interactions, such as electrostatic and Van der Waals forces, while adhesion to biotic surfaces is controlled by specific molecular mechanisms [17].

It was found experimentally that bacterial adhesion itself can be characterized by three parameters relating to [18-21]:

- bacteria (strain, bacterial growth, nutritional conditions, surface charge and energy)
- material surface (chemical composition, surface charge and energy, topology)
- surrounding environment (pH, temperature, presence of oxygen and other organic and inorganic compounds, hydrodynamic parameters)

3.2. Biofilm formation

In spite of the absence of a theory fully clarifying the initial phase of bacterial surface adhesion, the process of biofilm formation is described in relative detail. It can be divided into five stages as indicated in Figure 3. This also contains surface pictures taken by a camera connected to an optical microscope. Figure 4 shows a detailed surface picture (taken by a scanning electron microscope) of a polymer-based catheter colonized by the bacterial strain *Staphylococcus aureus*.

1. **Initial stage** – also called *the conditioning phase*. Initially, reversible bacteria-surface interactions are predominant. However, traces of extracellular substances remain on the surface after the detachment of bacteria. It is believed that such residua play an important role in the following stage of biofilm formation.
2. **Stage of irreversible attachment** – (primary adhesion); the predominant occurrence of stable bacterial attachment on the surface.
3. **Formation of micro-colonies** - (maturation phase 1); attached microorganisms reproduce and form colonies. The kinetics of cell division can, under ideal conditions, be expressed as " $m2^n$ ", where m is the number of colonies at the beginning and n represents the number of generations [16].
4. **Biofilm formation** – (maturation phase 2); adhered cells (microorganisms) produce extracellular compounds (often based on polysaccharides). Eventually the whole surface can be covered by extracellular secretion – the biofilm is formed. The environment of the biofilm is ideal for further reproduction of microorganisms. It was found that a lethal dose of antibiotics is more than one hundred times greater for bacteria present in the biofilm in comparison with their freely floating counterparts [22].
5. **Distribution stage** – the growing mass of biofilm leads to it rupturing. The bacteria present spread into the surrounding environment – i.e. the human body in the case of medical devices.

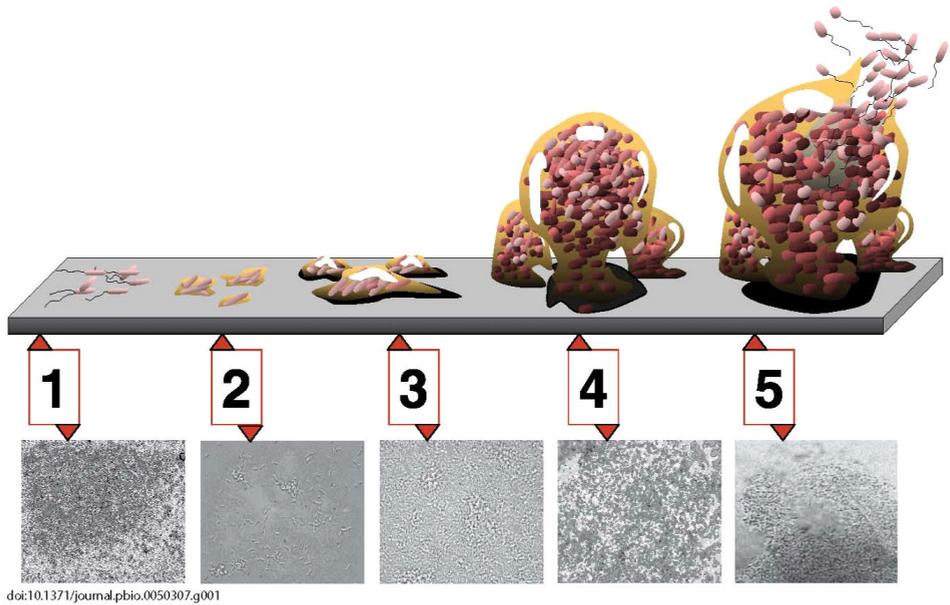


Figure 3. Five stages of biofilm formation (adopted from Monroe, D. doi:10.1371/journal.pbio.0050307 [22])

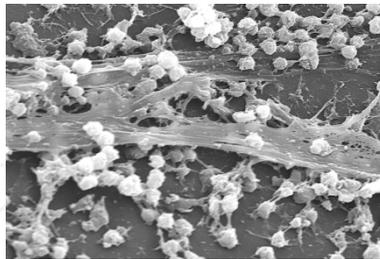


Figure 4. SEM picture of a plastic catheter colonized by *Staphylococcus aureus* (adopted from Monroe, D. doi: 10.1371/journal.pbio.0050307 [22])

Slight differences in describing the biofilm formation process can be found in the literature. However, these discrepancies are insignificant from the principle presented here. Semi-empirical models explaining the kinetics of bacterial surface adsorption and desorption have been developed. These calculations are applied, for example, in the spheres of waste water treatment and biotechnology. However, such processes are too dissimilar in comparison with

the colonization of medical devices (e.g. urinary catheters) to be mentioned in this work. Nevertheless, they can be found in the relevant literature [14, 16, 23, 24].

4. Antimicrobial modifications of polymers

An antimicrobial polymer system is a material intentionally modified (chemically or physically) to prevent its bacterial colonization. It usually consists of a polymer matrix and an antimicrobial agent. Antimicrobial polymers have been applied in medicine, food packaging as well as in the personal hygiene industry.

Strictly speaking there are four principles for antimicrobial modification of polymers.

Any selection of such a modification method should consider the following factors [16, 25, 26]:

- polymer properties (chemical and physical)
- intended use (humidity, temperature, pH factors, etc.)
- characteristics of the antimicrobial agent (toxicity, thermal stability, affinity with a certain component)
- technological factors (complexity, functionality, reproducibility)
- financial factors (financial burden versus added value, certification)

The majority of medical devices are made from a few known types of polymers (see Table 1). Beside these, a group of special polymers should be highlighted here as well. Special polymers are used as, for example, a hydrophilic coating layer (e.g. polyvinyl alcohol, polyvinylpyrrolidone), and bioactive substance carriers, etc.

a. Polymer modification without an antimicrobial compound

This technique originates from the basic assumption that modifying the surface properties of a material (surface free energy, polarity, topography) may result in diminishing bacterial adhesion during the initial stage of the biofilm formation process.

Modification can be performed either by applying wet chemistry through reaction with various chemical reagents, or by applying high energy electromagnetic radiation (e.g. by laser, ultraviolet radiation, gamma rays). The interaction of a polymer's surface with electromagnetic radiation causes surface activation (through the breakage of accessible polymer bonds), permitting subsequent chemical modification [27-29]. Another promising method is modifying polymer surfaces by ionized gas (plasma). This leads, naturally, to the selection of so-called *cold plasma* when the temperature of the treated material does not reach high figures in comparison with the ambient temperature. This method demands low pressure (0.1 – 100 Pa) and the presence of a working gas (usually N₂, O₂ or Ar, CF₄). Plasma modifications to polymer surfaces are characterized by their weak stability over time, as polymer surfaces tend to return to their original chemical state [30-40].

The extent of surface modification is a measure of free surface energy change, which can be simply detected by a contact angle technique, based on wettability by selected liquid determination. In the case of water, one measures the water contact angle (WCA).

The practical results of such surface modification are shown in Table 2.

In this experiment, the polymer surfaces were modified by various functional groups, which caused a dramatic change in WCA. These surfaces were subsequently tested for resistance to colonization by two bacterial strains (*Staphylococcus epidermidis* and *Deleya marina*). The results displayed in Table 2 represent the measure of bacterial colonization (100% stands for no effect in comparison with the unmodified surface). In this particular instance it is noticeable that a surface modification resulting in the WCA value 35° is optimal for reducing both strains. However, there is no universal surface modification technique that could prove applicable against all bacterial strains due to the rich diversity of microorganisms. Another disadvantage lies in the complexity of the surface modification methods.

Modification	WCA (°)	Colonization	
		<i>Staphylococcus epidermidis</i> (%)	<i>Deleya marina</i> (%)
$-(\text{CH}_2)(\text{CF}_2)_7\text{CF}_3$	120	27	49
$-(\text{CH}_2)_{15}\text{CH}_3$	107	57	100
$-(\text{CH}_2)_{11}(\text{OCH}_2\text{CH}_2)_6$	35	0.3	0.3
$-(\text{CH}_2)_{15}\text{COOH}$	< 5	100	23

Table 2. Effect of polymer surface modification on the extent of bacterial colonization [25]

b. Method for direct deposition of an antimicrobial agent on a polymer surface

This represents the simplest technique, one widely used in medical practice. An antimicrobial agent is applied to the surface of a polymer-based medical device just prior to use. The antimicrobial agent is usually in the form of a solution or ointment. However, low efficiency, caused by fast resorption of the active component, is the disadvantage of this method [41].

c. Method for surface modification and chemical deposition of an antimicrobial agent

Here the antimicrobial agent is chemically deposited either on the surface of the polymer (after prior activation) or by way of a relevant mediator (often based on polyacrylic acid). The mediator is grafted on the polymer surface and it forms a polymer brush (see Figure 5). The ends of the mediator chains can be used to immobilize the antimicrobial component. The function is similar to the previous case – the antimicrobial agent is released from the surface and deactivates any potentially present bacteria. The advantage of this technique lies in its activity over a long period of time [39, 42, 43]. Nevertheless, the complexity of the modification

process can prove limiting for this method. The issue of the mechanical stability of the thin surface layers should be also considered before opting for a modification technique.

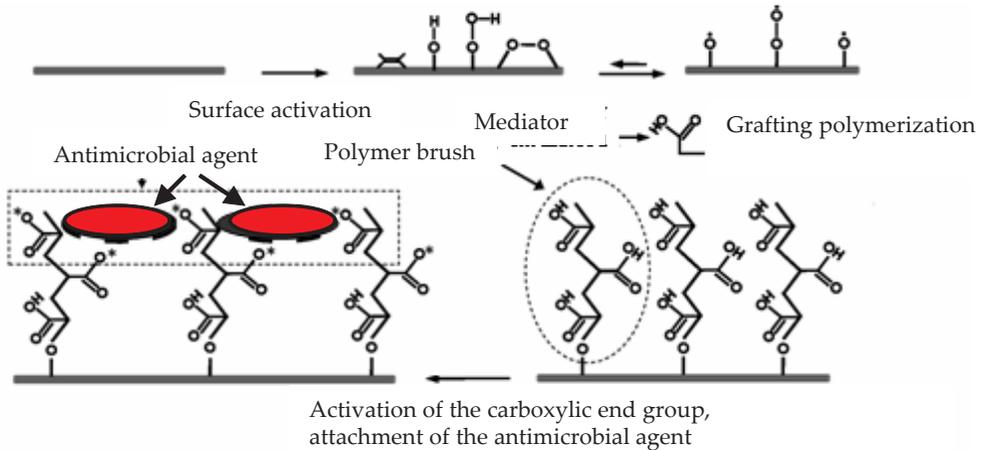


Figure 5. Schematic representation of antimicrobial agent immobilization on a polymer surface mediated by a polymer brush [43]

d. Method for bulk modification of a polymer with an antimicrobial agent

The last technique listed of antimicrobial polymer modification methods is based on directly incorporating the antimicrobial agent in a polymer matrix. This can be carried out in two ways. The first is suitable for preparing special coatings and films when mass production is not anticipated. The antimicrobial agent is introduced into a polymer solution that is cast subsequently. A dipping technique can be used in the case of coatings. The second way is applicable for large-scale production using thermoplastic polymer matrices. The antimicrobial agent is mixed with a polymer melt and processed by conventional techniques (extrusion, injection molding, blow molding, etc.).

Both methods for bulk modification of polymers are not technologically demanding. The antimicrobial additives behave in analogy with polymer fillers. The concentration of the antimicrobial agent in the polymer matrix does not usually exceed 20 vol. %.

A definite advantage of this method is the fact that, in most cases, the processing parameters as well as the technology necessary do not require significant modification. Moreover, with a lower additive content, the resulting mechanical properties of the modified materials are similar to those of the unmodified polymer matrix. However, the efficiency of antimicrobial modification (related to the amount of the incorporated agent) is low due to the restricted diffusibility of the antimicrobial agent molecules through the polymer matrix. It leads to most of the incorporated agent getting trapped in the polymer matrix, which cannot then become involved in the antimicrobial process. When designing a product, it is necessary to carefully select the antimicrobial agent (stability, efficiency, and economy), the processing techniques

(bulk modification versus thin coating layers) and the parameters (temperature stability of an additive) [44, 45].

Nowadays, antimicrobial polymer additives are available commercially. They are designed for various types of polymer matrices and processing techniques. These additives are often based on organic compounds or some metals (Ag, Zn, Cu, etc.) [5, 46, 47]. However, it should be noted that only a marginal number of them are considered for medical use.

The pros and cons of the introduced method for antimicrobial polymer modification are summarized in Table 3.

Method	Principle	Pros	Cons
a)	Modification of polymer surface properties without an antimicrobial agent	Use of chemicals is avoided	Generally ineffective, technologically demanding
b)	Direct deposition of the antimicrobial agent on the polymer surface	Cheap, simple, fast	Low efficiency
c)	Chemical deposition of the antimicrobial agent on the polymer surface	Limited amount of the antimicrobial agent is required	Technologically demanding, expensive
d)	Direct incorporation of the antimicrobial agent in the polymer matrix	Simple, possible to prepare using conventional technology	Low efficiency, high concentration of antimicrobial agent is required, limitations as regards temperature stability in thermoplastic processing

Table 3. Summary of antimicrobial modification methods

5. Evaluation of antimicrobial properties

A methodology for evaluating antimicrobial activity is necessary from the viewpoint of material safety as well as to verify the efficiency of the antimicrobial modification process.

Historically, the fundamentals of antimicrobial testing methodology were developed for the purposes of the textile industry [48]. The standards for antimicrobial testing of textiles are well formulated as a consequence. A growing interest in polymer materials and optimizing their properties for more complex applications brought about the need to modify existing standards to make them applicable to polymers as well.

5.1. Testing the antimicrobial properties of polymer-based materials

The subheading reveals that testing antimicrobial properties involves observing the biological activity of microbes (mostly bacteria and mould). This means their ability to survive an effect

of a given chemical compound at a certain concentration and for a certain time period. As regards terminology, resistance to bacteria is termed antibacterial, and to mould is termed antimicrobial. The general word covering all microorganisms is antimicrobial. In principle, methods for evaluating antimicrobial activity can be divided into two groups: (a) static methods and (b) dynamic methods [49].

a. Static methods for antimicrobial testing

- Microscopy (optical, electron, fluorescence) – bacteria counting, morphology observation
- Survival of bacteria detection – observing growth inhibition, determining the number of colony forming units (CFU), special marking techniques
- Biofilm evaluation

The number of colony forming units (CFU) is usually related to a certain volume or area (in the case of plastic films), and it is the most commonly used parameter in an antimicrobial testing procedure. Other indicators (however less frequently used) are connected with observing microbial metabolic activity through detection with relevant enzymatic apparatus, products of substrate metabolization, or observing consumption of another component (e.g. oxygen). A special approach can be found in a test dedicated to evaluating bacterial adhesion on selected surfaces where a special procedure for sample preparation must be applied prior to microbiological investigation. The microorganisms obtained from isolated strains or, if originating in real conditions, can be used as an inoculum.

In the case of biofilm testing via static methods, a sample after cultivation under given conditions (selected in accordance with standard or real conditions) goes through specific procedures, which involves washing and mechanical removal of irreversibly attached cells. Some research papers state various intensity levels of mechanical removal (from classical mixing to ultrasound application). Finally, the amount of removed bacteria can be simply determined by dilution and the spread plate technique. The results of such an experiment are shown in Figure 6b.

Testing antimicrobial activity on agar plates (the Agar diffusion test or the Kirby-Bauer disk-diffusion method) provides semi-quantitative information on the diffusibility of the antimicrobial agent from the specimen, and its efficiency against the given microorganism. After a chosen period of incubation, the area of bacteria growth is observed (see Fig. 6a).

Other (not as experimentally demanding as cultivation procedure) techniques for bacterial growth/inhibition detection are based on the fact that the presence of bacteria in a medium affects its properties. One of them is turbidity, which is directly proportional to the number of CFU; i.e. the quantity of CFU present in a cultivation medium can be determined by a spectroscopic method against a calibration curve [50]. Unfortunately, this considers both living and dead cells, which can significantly influence the result. A relatively new method is an ATP assay, which permits relatively rapid determination of the CFU number [51].

1. Dynamic methods for antimicrobial testing

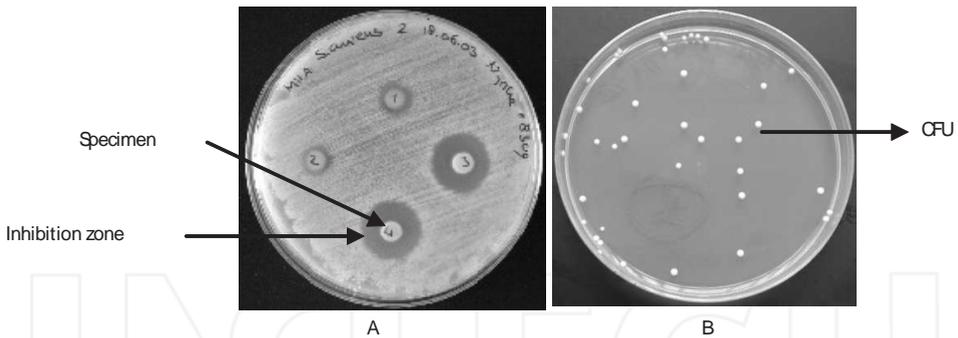


Figure 6. Outputs of antimicrobial testing (A) Agar diffusion test, (B) Dilution and spread plate technique test

This group of methods is based on measuring the flow characteristics of bacterial suspensions. The concentration of microorganisms in a given liquid medium is related to its viscosity. Dynamic methods can be divided, according the arrangement of the experiment, as follows [14]:

- Parallel plate flow system – the upper plate is made from glass while the lower part is made from the tested material. The flow of the liquid medium generates shear stress on the walls (τ_w), which can be calculated, providing values of pressure change (ΔP) and the dimensions of the channel (height (h) and width (L)) are known [52]:

$$\tau_w = \frac{\Delta P h}{2L} \quad (1)$$

- Measurement on a radial flow cell – the system consists of two concentric disks. The upper one of is made from transparent materials, while the lower one is made from the tested material. For a given flow (Q), the shear rate on the surface of the tested sample is inversely related to disk radius (r) and the gap between the discs (h) [53]:

$$S = \frac{3Q}{\pi r h^2} \quad (2)$$

- Measurement by rotating disc – this can be used in both the laminar and turbulent region of flow [54].

A schematic overview of antimicrobial testing methods is shown in Figure 7.

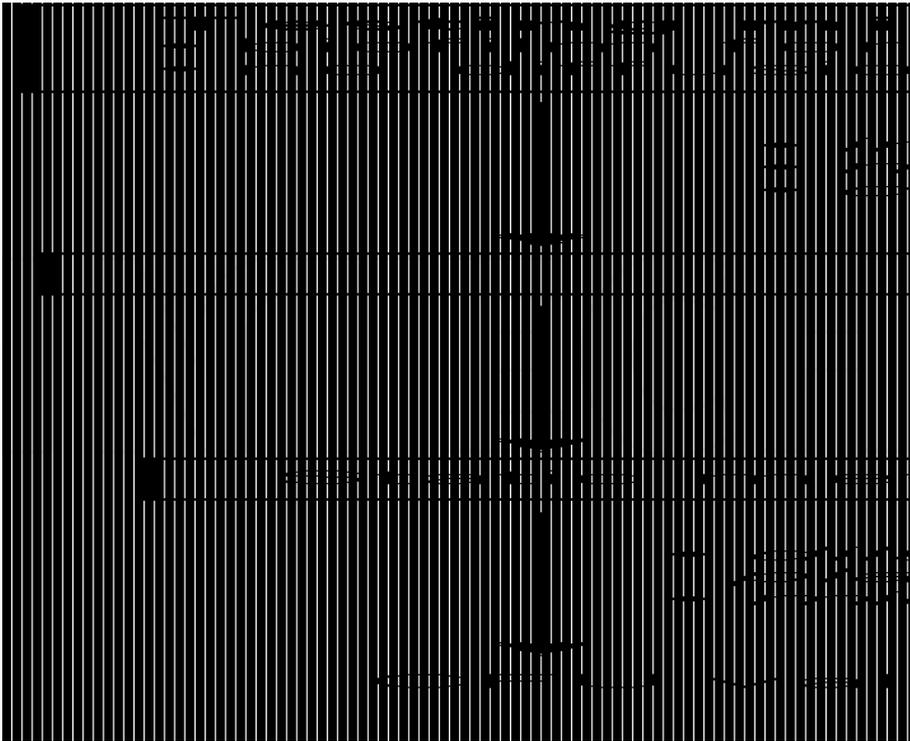


Figure 7. Schematic overview of the antimicrobial testing of polymer-based materials

5.2. Selected standards relating to the antimicrobial testing of materials

- ISO 22196:2011 “Measurement on the antibacterial activity of plastic and other non-porous surfaces”
- ASTM E2180-07 “Standard Test Method for Determining the Activity of Incorporated Antimicrobial Agent(s) In Polymeric or Hydrophobic Materials”
- ASTM E2149-10 “Standard Test Method for Determining the Antimicrobial Activity of Immobilized Antimicrobial Agents under Dynamic Contact Conditions”
- ASTM G21-09 “Standard Practice for Determining the Resistance of Synthetic Polymeric Materials to Fungi”
- ISO/CD 16256 “Clinical Laboratory Testing and In Vitro Diagnostic Systems – Reference Method for Testing the In Vitro Activity of Antimicrobial Agents Against Yeast Fungi Involved in Infectious Diseases”
- ISO 20645:2004 “Textile Fabrics -- Determination of Antibacterial Activity - Agar Diffusion Plate Test”

6. Conclusions

The bacterial colonization of surfaces, by subsequent biofilm formation on polymer-based medical devices, are the cause of serious health complications mediated by nosocomial infections. In addition, treating these infections gives rise to significant financial burden to health care systems. Applying antimicrobial polymers can noticeably reduce this negative phenomenon.

This text has provided an overview of the principles of bacterial adhesion and the biofilm formation process. General methods for antimicrobial modification of polymers have been discussed in accordance with current scientific works. Furthermore, techniques for antimicrobial properties testing have been introduced and summarized.

Research in the field of antimicrobial polymers is progressing rapidly due to intense demand for practical applications. Nevertheless, the overuse of antimicrobial agents should not occur, so as to avoid the occurrence of microbial resistance and environmental damage.

Acknowledgements

This work was financially supported by the Ministry of Education, Youth and Sports of the Czech Republic and Operational Program Research and Development for Innovations co-funded by the European Regional Development Fund (ERDF, project No. CZ. 1.05/2.1.00/03.0111).

Author details

Vladimir Sedlarik^{1,2*}

1 Centre of Polymer Systems, University Institute, Tomas Bata University in Zlin, Zlin, Czech Republic

2 Polymer Centre, Faculty of Technology, Tomas Bata University in Zlin, Zlin, Czech Republic

References

- [1] Scott, G. Green polymers. *Polymer Degradation and Stability* (2000). , 68-1.
- [2] Scott, G, & Gilead, D. *Degradable Polymers: Principles and Applications*. Dordrecht: Kluwer Academic Publishers/Chapman and Hall; (1995).

- [3] Zhang, W, Ji, J, Zhang, Y, Yan, Q, Kurmaev, E. Z, Moewes, A, Zhao, J, & Chu, P. K. Effect of NH₃, O₂ and N₂ Co-Implantation on Cu out Diffusion and Antimicrobial Properties of Copper Plasma-Implanted Polyethylene. *Applied Surface Science* (2007). , 2007, 253-8991.
- [4] Buschow, K. H. J, Cahn, R. W, Flemings, R. C, Ilschnr, B, Kramer, E. J, & Mahajan, S. *Encyclopedia of Materials- Science and Technology*. Amsterdam: Elsevier; (2001).
- [5] Galya, T. *Antibacterial Modifications of Polymers by Using Metallic Compounds*, Doctoral thesis, Zlin: Tomas Bata University in Zlin; (2009).
- [6] Xu, X, Yang, Q, Wang, Y, Yu, H, Chen, X, & Jing, X. Biodegradable electrospun poly(L-lactide) fibres containg antibacterial silver nanoparticles. *European Polymer Journal* (2006). , 2006, 42-2081.
- [7] Woo, G. L. Y, Mittelman, M. W, & Santere, J. P. Synthesis and Characterization of Novel Biodegradable and Antimicrobial Polymer. *Biomaterials* (2000). , 2000, 21-1235.
- [8] DuceL, G, Fabry, J, & Nicolle, L. *Prevention of Hospital Acquired Infections- Practical Guide*. Document of World Health Organization WHO/CDS/EPH/2002.12; (2002).
- [9] Pace, L. J, Rupp, M. E, & Finch, R. G. *Biofilms, Infection, and Antimicrobial Therapy*. Boca Raton: CRC Press Taylor&Francis Group; (2006).
- [10] Marshall, K. C. *Mechanisms of Bacterial Adhesion at Solid-Water Interfaces*, published in *Bacterial Adhesion*, Savage G.C., Fletcher, M (eds) New York: Plenum Press; (1985).
- [11] Wiencek, K. M, & Fletcher, M. Bacterial adhesion to hydroxyl-an Methyl-Terminated Alkanethiol Self-Assembled Monolayers. *Journal of Bacteriology* (1995). , 1995, 177-1959.
- [12] Fletcher, M, & Loeb, G. I. Influence of Substratum Characteristics on the Attachment of a Marine Pseudomonad to Solid Surfaces. *Applied and Environmental Microbiology* (1979). , 1979, 37-67.
- [13] Morra, M, & Cassinelli, C. Bacterial Adhesion to Polymer Surfaces: A Critical Review of Surface Thermodynamic Approaches. *Journal of Biomaterial Science, Polymer Edition* (1997). , 1997, 9-55.
- [14] Katsikogianni, M, & Missirlis, Y. F. Concise Review of Mechanisms of Bacterial Adhesion to Biomaterials and of Techniques Estimating Bacteria Material Interactions. *European Cells and Materials* (2004). , 2004, 8-37.
- [15] Verwey, E. J. W, & Overbeek, J. T. G. *Theory of the Stability of Lyophobic Colloids*. Amsterdam: Elsevier; (1948).
- [16] Ratter, B. D, Hoffmann, A. S, Schoen, F. J, & Lemons, J. E. *Biomaterials Science- An Introduction to Materials in Medicine*. London: Elsevier Academic Press; (2004).

- [17] Heilman, C, Schweitzer, O, Gerke, C, Vanittanakom, N, Mack, D, & Gotz, F. Molecular Basis of Intercellular Adhesion in the Biofilm-forming *Staphylococcus epidermidis*. *Molecular Microbiology* (1996). , 1996, 20-1083.
- [18] An, Y. H, & Friedman, R. J. Cencise Review of Mechanisms of Bacterial Adhesion to Biomaterial Surfaces. *Journal of Biomedical Materials Research* (1998). , 1998, 43-338.
- [19] Cordenro, J, Munuera, L, & Folgueira, M. D. The Influence of the Chemical Composition on Surface of the Implant Infection. *Injury* (1996). , 1996, 27-34.
- [20] Balasz, D. J, Triandafillu, K, Chevolut, Y, Aronsson, B. O, Harms, H, Descouts, P, & Mathieu, H. J. Surface modification of PVC Endotracheal Tubes by Oxygen Glow Discharge to Reduce Bacterial Adhesion. *Surface and Interface Analysis* (2003). , 2003, 35-301.
- [21] Vacheethasanee, K, Temenhoff, J. J, Higashi, J. M, Gary, A, Aanderson, J. M, Bayston, R, & Marchant, R. E. Bacterial Surface Properties of Clinically Isolated *Staphylococcus epidermidis* Strains Determine Adhesion on Polyethylene. *Journal of Materials Research* (1998). , 1998, 42-425.
- [22] Monroe, D. Looking for Chinks in the Amour of Bacterial Biofilms. *PLoS Biology* (2007). , 2007, 5-2458.
- [23] Anonymous Microbial Degradation of Materials and Methods of Protection, Published for the European Federation of Corrosion, The Institute of Materials, (1992). ISBN 0-901716-02-2
- [24] Blashek, H. P, Wang, H. H, & Agle, M. E. *Biofilms in the Food Environment*. Oxford: IFT Press-Bllackwell Publishing; (2007).
- [25] Wong, W. Y, & Brozino, J. D. *Biomaterials*. Boca Raton: CRC Press; (2007).
- [26] Hin, T. S. *Engineering of Materials for Biomedical Applications*. New Jersey: World Scientific; (2004).
- [27] Piccirillo, C, Perni, S, Cil-thomas, J, Prokopovich, P, Wilson, M, Pratten, J, & Parkin, I. P. Antimicrobial Activity of Methylene Blue Covalently Bound to a Modified Silicon Polymer Surface. *Journal of Materials Science* (2009). , 2009, 19-6167.
- [28] Gozzelino, G, Dellaquila, A. G, & Romero, D. Hygienic Coatings by UV Curing of Diacrylic Oligomers with Added Triclosan. *Journal of Coating Technology and Research* (2010). , 2010, 7-167.
- [29] Alvarez-Lorenzo, C, Bucio, E, Burillo, G, & Concheiro, A. Medical Devices Modified at The Surface by gamma-ray Grafting for Drug Loading and Delivery. *Expert Opinion on Drug Delivery* (2010). , 2010, 7-173.
- [30] North, S. H, Lock, E. H, Coper, C. J, Franek, J. B, Taitt, C. R, & Walton, S. G. Plasma-Based Surface Modification of Polystyrene Microtiter Plates for Covalent Immobilization of Biomacromolecules. *ACS Applied Materials & Interfaces* (2010). , 2010, 2-2884.

- [31] Asanovic, K, Mahailovic, T, Skundricic, P, & Simovic, L. Some Properties of Antimicrobial Coated Knitted Textile Material Evaluation. *Textile Research Journal* (2010). , 2010, 80-1665.
- [32] Cheruthazhekatt, S, Cermak, M, Slavicek, P, & Havel, J. Gas Plasmas and Plasma Modified Materials in Medicine. *Journal of Applied Biomedicine* (2010). , 2010, 8-55.
- [33] Tseng, H. J, Hsu, S. H, Wu, M. W, Hsen, T. H, & Tu, P. C. Nylon Textiles Grafted with Chitosan by Open Air Plasma and their Antimicrobial Effect. *Fibers and Polymers* (2009). , 2009, 10-53.
- [34] Jampala, S. N, Sarmadi, M, Somers, E. B, Wong, A. C. L, & Denes, F. S. Plasma- Enhanced Synthesis of Bactericidal Quaternary Ammonium Thin Layers on Stainless Steel and Cellulose Surfaces. *Langmuir* (2008). , 2008(24), 8583-8591.
- [35] Gomathi, N, Surehkumar, A, Neogi, S, & Plasma-treated, R. F. *Polymers for Biomedicam Applications. Current Science* (2008). , 2008, 94-1478.
- [36] Abdou, E. S, Elkholy, S. S, Elsabee, M. Z, & Mohamed, E. Improved Antimicrobial Activity of Polypropylene and Cotton Nonwoven Fabrics by Surface Treatment and Modification with Chitosan. *Journa of Applied Polymer Science* (2008). , 2008, 108-2290.
- [37] Zhang, W, Zhang, Y. H, Ji, J. H, Yan, Q, Huang, A. P, & Chul, P. K. Antimicrobial Polyethylene with Controlled Copper Release, *Journal of Biomedical Materials Research A* (2007). A , 838-844.
- [38] Asandulesa, M, Topala, I, & Dimitrascu, N. Effect of Helium DBD Plasma Treatment on the Surface of Wood Samples. *Holzforschung* (2010). , 2010, 64-223.
- [39] Asadinezhad, A, Novak, I, Lehocky, M, Sedlarik, V, Vesel, A, Junkar, I, Saha, P, & Chodak, I. An in Vitro Bacterial Adhesion Assesment of Surface-Modified Medical Grafe PVC. *Colloids and Surfaces B- Bointerfaces* (2010). , 2010, 77-245.
- [40] Zhang, W, Chu, P. K, Zhang, Y, Fu, H, & Yan, R. K. Y. Q. Antibacterial properties of Plasma Modified and Triclosan or Bronopol Coated Polyethylene. *Polymer* (2006). , 2006, 47-931.
- [41] Laporte, R. J. *Hydrophilic Polymer Coatings for Medical Devices-Structure, Properties, Development, Manufacture and Application*. Boca Raton: CRC Press (1997).
- [42] Asadinezhad, A, Novak, I, Lehocky, M, Bilek, F, Vesel, A, Junkar, I, Saha, P, & Popelka, A. Polysaccharides Coatings on Medical-Grade PVC: A Probe Into Surface Characteristics and the Extent of Bacterial Adhesion. *Molecules* (2010). , 2010, 15-1007.
- [43] Asadineshad, A, Novak, I, Lehocky, M, Sedlarik, V, Vesel, A, Junkar, I, Saha, P, & Chodak, I. A Physicochemical Approach to render Antibacterial Surfaces on Plasma-Treated Medical-Grade PVC: Irganon Coating. *Plasma Processes and Polymers* (2010). , 2010(7), 504-514.

- [44] Ash, I, & Ash, M. Handbook of Preservatives. Endicott: Synapse Information Resources Inc.; (2009).
- [45] Paulus, W. Directory of Herbicides for the Protection of Materials- A Handbook. Dordrecht: Springer; (2005).
- [46] Radeshkumar, C, & Munstedt, H. Antimicrobial Polymers from Polypropylene/Silver Composites-Ag⁺ Release Measured by Anode Stripping Voltametry. Reactive & Functional Polymers (2006). , 2006, 66-780.
- [47] Radeshkumar, C, & Munstedt, H. Morphology and Mechanical Properties of Antimicrobial Polyamide/Silver Composites. Material Letters (2005). , 2005, 59-1945.
- [48] Jakimiak, B, Rohm-rodowald, E, Stanieszewska, M, Cieslak, M, Malinowska, G, & Kaleta, A. Microbial Assessment of Efficiency of Antimicrobial Modified Textiles. Rocznik Panstwowego Zakladu Higieny (2006). , 2006, 57-177.
- [49] An, Y. H, & Friedman, R. J. Laboratory Methods for Studies of Bacterial Adhesion. Journal of Microbiological Methods (1997). , 1997, 30-141.
- [50] Sutton, S. Determination of Inoculum for Microbial Testing. Microbiology Topics (2011). , 2011, 15-49.
- [51] Baoshan, H, Xiaohong, L, Weiwei, Y, Aiyu, Z, Jinping, L, & Xinxia, C. Rapid detection of bacteria without cultivation with a portable bioluminescence sensor system. African Journal of Microbiology Research (2009). , 2009, 3-575.
- [52] Bakker, D. P, Huijs, F. M, Devries, J, Klijnsstra, J. W, Vusscher, H. H, & Van Der Mei, H. C. Bacterial deposition to fluoridated and non-fluoridated polyurethane coatings with different elastic modulus and surface tension in a parallel plate and a stagnation point flow chamber. Colloids and Surfaces B- Biointerfaces (2003). , 2003, 32-179.
- [53] Dickinson, R. B, Nagel, J. A, Mcdevitt, D, Foster, T. J, Proctor, R. A, & Cooper, S. L. Quantitative comparison of clumping factor- and coagulase mediated *Staphylococcus aureus* adhesion to surface-bound fibrinogen under flow. Infection and Immunity (1995). , 1995, 63-3143.
- [54] Dejong, P, Tegiffel, M. C, & Kiezebrink, E. A. Prediction of the adherence, growth and release of microorganisms in production chains. International Journal of Food Microbiology (2002). , 2002, 74-13.