Tomas Bata University in Zlín
Faculty of Technology

Doctoral Thesis

Bioactive polymer surfaces based on polyethylene

Bioaktivní polymerní povrchy na bázi polyethylenu

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ABSTRACT

Polymeric biomaterials are widely used besides metals, ceramics and composite biomaterials due to their easy processability, low cost and favourable physical and chemical properties. However, their surface properties does not often meet optimal level. Due to this fact, the surface modification of polymer materials used as a medical devices is of a paramount importance during the processing. Such modification can be done via several available methods. Besides flame treatment, UV irradiation and wet chemical etching by strong acids, the most advantageous method is plasma surface modification both from economical point of view and is environmental friendly. Further processing of such modified material leads to the creation of layer with bioactive and intelligent properties in terms of the interaction between the synthesized biomaterial and cells.

ABSTRAKT

Polymerní biomateriály jsou široce používány vedle kovů, keramiky a kompozitmích biomateriálů pro jejich jednoduchou zpracovatelnost, nízké náklady a vhodné fyzikální a chemické vlastnosti. Přes to, jejich povrchové vlastnosti nejsou na optimální úrovni. V důsledku toho hraje povrchová úprava polymerních materiálů podstatnou roli při jejich zpracování. Vedle opracování plamenem, UV ozařováním a leptáním mokrou cestou pomocí silných kyselin je nejvýhodnější metodou povrchová modifikace v plazmatu jak z ekonomického hlediska, tak i z hlediska ekologické šetrnosti. Další zpracování takto modifikovaných materiálů vede k tvorbě vrstvy bioaktivního a inteligentního materiálu, který vhodným způsobem interaguje s buňkami.

1. THEORETICAL BACKGROUND

1.1 Biomaterials

Biomaterials are the materials used in applications where they interact with the biological systems, mainly in the human body in order to replace or treat soft/hard tissues, and also extensively used in pharmaceutical area and medical diagnostic devices and supplies. It's a highly interdisciplinary research area included a knowledge of materials science and engineering, tissue engineering, medicine, physics, chemistry and biology. First generation of the biomaterials was inert materials with an interest of toxicity; bioactivity was the target of second generation of the biomaterials; and regeneration of tissue is the interest of third generation of the biomaterials [1]. According to the needs in biomedical applications, all three generation of the biomaterials are still in use today.

Classification of biomaterials can be expressed as metals (stainless steel, titanium alloys, Co-Cr alloys, etc), ceramics (calcium phosphate, aluminium oxide), polymers and composites. Metals are mostly used as orthopedic implants due to their tension-compression strength; ceramics are used in dentistry or hip-joint implants, where high compression strength needed. Owing to mechanical properties of polymeric materials vary in a broad range, their use in medical application also varies. Finally, composite biomaterials have great interest due to combination of advantages of several materials. Some of the specific applications may need combination of both ceramic, metallic and polymeric materials, such in hip joints (Fig.1.1).

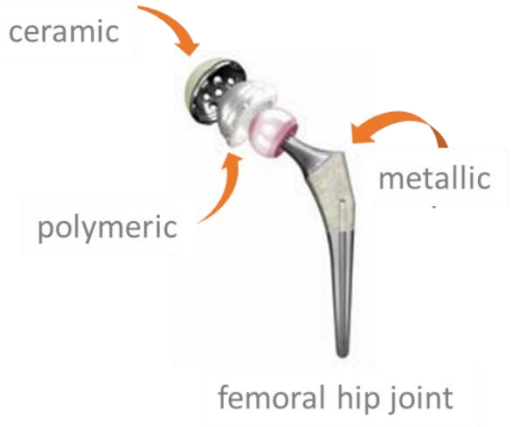

Fig. 1-1: Femoral hip joint consist of ceramic, metallic and polymeric materials. [http://www.stryker.com/]

Biomaterials have broad range of medical applications, such as orthopedic and dental implants, heart valves, joint replacements, bone plate/cements, blood vessel prostheses, coronary stents, artificial ligaments/tendons, skin replacements and contact lenses [2].

Selection of biomaterials varies according to their specific medical application, therefore it depends on the mechanical properties (such as hardness, tensile/compression strength, viscosity), fatigue resistance, corrosion resistance, wear resistance, elastic modulus, permeability, processability, optical properties, weight and costs [3].

Besides initial bulk properties of the biomaterials, their surface finish properties, such as surface chemistry, tension, roughness, wettability have paramount importance for surface-living system interactions which is the interest of this research.

1.2 A brief overview of polymers

Polymers are long-chain giant macromolecules, consist of macromolecular organic (carbon based) compounds, either naturally occurring natural polymers (cellulose, starch, horn, hair, etc) or synthetically formed synthetic polymers, also known as plastics [4,5]. These macromolecules (polymers) consist of repeating 'mer' units of a monomer (one unit), which called as homopolymers; or different monomers, which called copolymers, as depicted in fig. 1.2. [4]. Each 'mer' units linked to each other by primary, covalent bonds by chemical reactions, called as polymerization [Young]. Polymerization process to link the small molecules, basically divided to two processes, namely condensation (or step growth) and addition (chain growth) polymerization (anionic and cationic) processes [7].

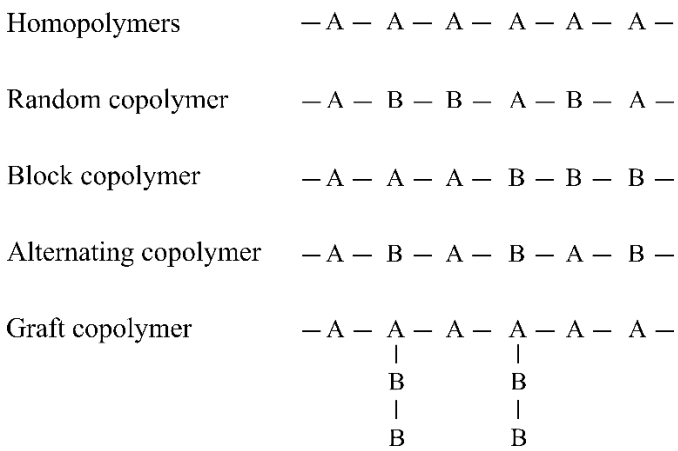

Fig. 1-2: Types of polymer according to its "mer" arrangement.

According to their molecular chain structure, they can be formed as linear, branched and crosslinked polymers [8] as it seen in Fig. 1.3. Linear polymers consist of the same repeating monomer units, joined together in a single chain (also called as backbone chain) side by side [8]. Some of the linear polymers are polyethylene (PE), polystyrene (PS), polyvinyl chloride (PVC), polymethyl methacrylate (PMMA), nylon 6-6 [8,9]. Branched polymers can be expressed as linear polymer with a side branch chains on their main backbone (Fig.2b). Due to its side chains lower the chain packing efficiency, density of the polymer is lowered, as an example of low density polyethylene (LDPE) [8]. Crosslinked polymers have linear chains joined to each other by covalent bonds [8]. According to polymerization conditions, each polymer chain have different numbers of 'mer' units and chain length. Average number of 'mers' or repeating units per molecule refers polymerization degree, which is related to molecular weight of a polymer plays important role on physical properties [9].

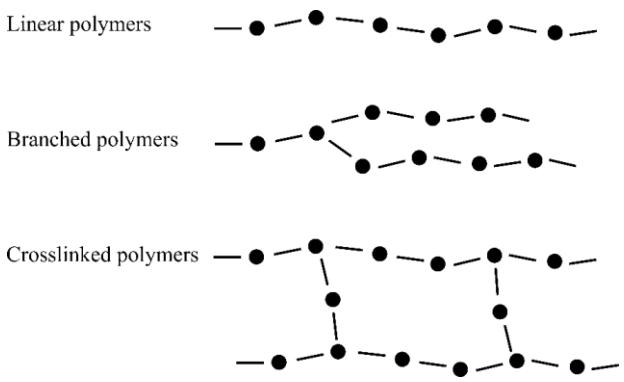

Fig. 1-3: Schematic representations of linear, branched and crosslinked polymers.

Natural polymers derived from either animals or plants naturally, such as cellulose, starch, proteins and enzymes [8]. Synthetic polymers, commonly called as plastics, which is produced by polymerizations processes have great attention on the market, especially after second world war [5,8]. Due to its lower cost, easy processability, sufficient mechanical properties and chemical stability, polymeric materials have been widely used for various applications according their commodity or performance, such as packaging, automotive industry, aeronautics, etc. [10,11].

According to thermal properties, synthetic polymers divided to two groups, thermoplastics and thermosets. Thermoplastics are able to hardening and softening by temperature due to their linear or branched chain structure, therefore it is possible to reshaped or recycle them. Thermosets are crosslinked polymers which have higher rigidity, therefore it is not possible to melt them back to re-shaped.

Polymeric materials are beneficial to reduction in cost, reduction in weight, corrosion resistance and insulation materials [12]. They can be produced by extrusion, moulding and thermoforming using their pellets.

1.3 Polymeric biomaterials

Polymeric materials have been widely used for various industrial applications such as packaging, automotive, aeronautics, electronics, corrosion resistance coating, insulation coating, light emitting devices due to their low cost, sufficient mechanical properties, chemical stability and easy processability, for years [10], [11]. Their usage in biomedical area have been exceptionally started during the World-War II by surgeons and since then gain attention in the biomedical research area to improve/create new polymers to meet the requirement of medical needs [13].

In the biomedical area (i.e. implants, wound dressing, suture, catheter, etc.) some of the natural and synthetic polymers are also widely used besides metals and ceramics as a polymeric biomaterials. The main advantages of polymeric biomaterials compared to metal and ceramics are low cost and easy processability, possibility of re-processability, better corrosion resistance, ease of production in specific shapes and offers versatility due to their carbon based organic structures [7,13,14]. Thus, their carbon based chemistry make them preferable materials than inorganic materials due to their interactions with the living tissues [5]. They are also preferable where the weight and/or elasticity is needed. Moreover, Material selection for polymeric biomaterials depends on the design considerations, physical and mechanical properties, thermal properties, chemical resistance, durability and sterilization capability [3]. Therefore, choosing an appropriate polymer for specific application, such mentioned properties of polymers, so then its physical and chemical behavior under special conditions, has to be well known for better match as far as possible. Such in other metallic and ceramic biomaterials, polymeric materials expected to be biocompatible as both mechanical and surface properties.

Natural polymers, also called as biopolymers, are produced by living organism and basically divided to three according to repeated monomer units of saccharide, amino acid or nucleic acid. They can be derive from plant and animal sources, such as storage polysaccharides of starch, glycogen or structural polysaccharides of cellulose, chitin, chitosan and bacterial polysaccharides. Amino acid based biopolymers are proteins and nucleic acid based biopolymers are ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). Thereby, enormous amount of source is exist to produce natural polymers to take advantage of their biodegradation. Such natural polymers derived from natural sources can be blend with synthetic polymers to create degradable polymers for specific applications, such as orthopedic fixation devices and ligament augmentation, which is able to degrade in time into the implanted body. Moreover, individual properties of natural polymers can bring in external benefits to blend polymer, such as chemical interactions with the living body, i.e. protein interactions, cellular interactions or bacterial interactions. Natural polymers is also useful materials to produce bio-

based non-degradable polymers, such as Bio-PET and Bio-PVC to take the advantage of enormous natural source to get rid of side effects of petrol based synthetic polymers and/or lower the cost. Besides, some of the synthetic polymers can undergo degradation by means of hydro-degradation and/or oxo-degradation, due to existence of their hydrolysable backbones (i.e., polyester, polyamide, polyurethane) or oxidisable backbones (i.e., polyvinyl alcohol, polyvinyl ester) , respectively. However, degradation of polymer used in medical application might be desired or non-desired condition depends on its application.

Synthetic polymeric biomaterials have found applications in various biomedical area, either for disposable and/or long term usage, such as in ophthalmology (i.e., contact lenses, intraocular lenses, artificial corneas, etc.); in orthopedics (i.e., bone fixation devices, load bearing implants, hip joints, knee joints, finger joints, dental implants, etc.); in cardiovascular diseases (i.e., vascular grafts, intra-aortic balloon pumps, artificial hearts, pacemakers, etc.) or extracorporeal devices, wound dressing materials, extracorporeal artificial organs, tissue engineered materials, encapsulants, drug delivery systems, nerve regeneration devices, medical disposable supply and pharmaceutical packaging, surgical gloves, metal and ceramics substituents, etc. [5,7,9,13].

Production of different kind of polymer for appropriate application basically depends on its monomer units, existence of co-polymers, polymerization reaction, therefore more closely meet the requirement of each application, listed in Table 1.1. For instance, polymers used in ophthalmology must be exhibit sufficient transmission of visible light, oxygen permeability, thermal conductivity, chemical stability, tear resistance and hydrophilicity [15]. Further, polymers used in orthopedics needs superior mechanical properties, such as tension/compression strength, hardness, wear resistance, fracture thoughness, etc. [5]. Last but not least, polymers used in cardiovascular purpose expected to exhibit more elasticity, tensile and fatigue strength, and adequate hemocompatibility.

Table 1.1. Most used synthetic polymers used in biomedical applications.

Polymers	**Biomedical application**
Poly(ethylene) (PE)	Containers, pharmaceutical packaging and bottles,non-wowen fabric, breather patches, artificial hip and knee joints, catheters, tubing drains, dentures, esophagus segments, heart pacemaker, etc.
Poly(propylene) (PP)	Suture materials, meshes, finger joint prostheses, disposable syringes, non-woven fabrics, artificial vascular grafts, blood oxygenator membrane, containers, medical trays, etc.
Poly(styrene) (PS)	Diagnostic devices, tissue culture components, vacuum canisters, filter wares, roller bottles, petri dishes, pipettes and laboratory wares, etc.
Polyurethane (PU)	Blood contacting devices, vascular grafts, heart assist balloon pumps, hemodialysis bloodlines, stents, artificial heart bladders, insulation pacemaker leads, etc.
Poly(vinyl chloride) (PVC)	Blood storage bags, extracorporeal tubings, catheter bottles, surgical packaging, dialysis devices, feeding, tubing, etc.
Polyamide (PA)	Suture materials, ligament and tendon repair materials, balloon of catheters and dialysis membranes.
Poly(methyl methacrylate) (PMMA)	Intraocular and hard contact lenses, bone cements, blood pump and reservoir, blood handling components, catheter, urological accessories, chest drainage unit, etc.
Poly(tetrafluoroethylene) (PTFE)	Vascular grafts, catheters, patches for hernia repair and surgical sutures.
Poly(dimethylsiloxane) (PDMS)	Blood vessels, heart valves, dialysis membranes, catheters, drainage tubing, finger joints, chin and nose implants, etc.
Poly(carbonate) (PC)	High pressure syringes, artery cannulas, insulin pens, glucose meters, luers, stopcocks, suture materials, dialysis membrane and containers, etc.
Poly(ethylene terephthalate) (PET)	Ligament and tendon repair materials, sutures, surgical meshes, vascular grafts, heart valves, etc.
Polyether sulfone (PES)	Dialysis membranes, fluid handling couplings and fittings, medical devices which needs repeated sterilization.
Polyether ether ketone (PEEK)	Orthopedic implant parts, inner lining of catheters, keyhole surgery devices, disposable surgical instruments, dental syringes, etc.

Frequently used synthetic polymers as polymeric biomaterials are roughly mentioned below, based on their chemical structure, production way, mechanical and chemical properties with mostly used biomedical applications.

1.3.1 Poly(ethylene)

Polyethylene (PE) is a polyolefin, which is very inert homo-polymer with a chemical formula of $(CH_2)_n$ and it has a hydrophobic nature. According to its production way, there are six types PE with a different molecular weights, defined by ASTM D1248 [5,9]. There are, ultra-low density polyethylene (ULDPE) with a density range of 0.890 – 0.905 g/cm^3; very-low density polyethylene (VLDPE) with a density range of 0.905 – 0.915 g/cm^3; linear-low density polyethylene (LLDPE) with a density range of 0.915 – 0.935 g/cm^3; Low-density polyethylene (LDPE) with a density range of 0.915 – 0.935 g/cm^3; medium density polyethylene (MDPE) with a density range of 0.926 – 0.940 g/cm^3; and cc with a density range of 0.940 – 0.970 g/cm^3 [7,13,16].

Each of them have different molecular weights due to their different production way (temperature, pressure, polymerization process, etc), therefore different crystallinity which differs their physical properties, ie. elastic modulus, hardness and strength [5]. For instance, LDPE is produced at high temperature range of 150-300 °C at high pressure of 1000 to 3000 kg/cm^2 using a free radical initiators to obtain highly branched polymer with a molecular weight of 50.000 – 200.000 and lower crystallinity of 40-50 %, therefore density [7]. LDPE is most soft PE among its counterparts with an elastic modulus of 100 – 500 MPa [5]. There is also ultra-high molecular weight polyethylene (UHMWPE) exist, with a molecular weight of 2.000.000 – 6.000.000 and crystallinity of 50-60 %, which can provide a high elastic modulus of 400 – 1500 MPa, refers high strength PE to use in load-bearing biomedical applications [5,13].

They found a wide potential use in biomedical applications, as containers, pharmaceutical packaging and bottles, non-wowen fabric, breather patches, artificial hip and knee joints, catheters, tubing drains, dentures, esophagus segments, heart pacemaker, etc. [5,7,9,16-18].

1.3.2 Poly(propylene)

Polypropylene (PP) is a biologically inert homo-polymer with a chemical formula of $(C_3H_6)_n$. PP belongs to polyolefin which show similar properties as PE. Compare to PE, it has higher stress cracking resistance with a high rigidity, appropriate tensile strength and chemical resistance [6,7,16].

It is also possible to create PP as random copolymer using a small amount of ethylene monomer to make them more flexible; as block copolymer using a higher amount of ethylene monomer to greater their impact resistance [9].

Applications of PP in biomedical area are suture materials, surgical meshes, finger joint prostheses, disposable syringes, non-woven fabrics, artificial vascular grafts, blood oxygenator membrane, containers, medical trays [5,7].

1.3.3 Poly(styrene)

Polystyrene (PS) is one of the most used, simplest plastic have limited flexibility with a chemical formula of $(C_8H_8)_n$ [9]. There are three grades of PS available in the market: General purpose of polystyrene (GPPS) which is unmodified with a relatively high elastic modulus; high impact polystyrene (HIPS) which contains a rubbery modifier to increase its impact strength and ductility; and foam form of polystyrene, which is also called as syndiotactic polystyrene (SPS) with oriented chemical structure [7,9].

PS commonly used as diagnostic devices, tissue culture components, vacuum canisters, filter wares, roller bottles, petri dishes, pipettes and laboratory wares [7,9].

1.3.4 Polyurethane

Polyurethane (PU) is a block copolymer with hard and soft blocks which makes them rubbery material, with a good fatigue resistance and excellent biocompatibility for blood-contacting material due to its protein adsorption properties [13,16]. Due to their unique morphology (containing soft and hard segments), PU have beneficial mechanical properties, i.e., according to amount of such segments, it can be rigid or elastomeric and it is highly stable [13].

Their biomedical applications are, blood contacting devices, vascular grafts, heart assist balloon pumps, hemodialysis bloodlines, stents, tubing, artificial heart bladders, insulation pacemaker leads [7,13,16].

1.3.5 Poly(vinyl chloride)

Polyvinyl chloride (PVC) is chemically inert polymer with ethylene backbone with the large side group of covalently bonded chloride, which makes them rigid and brittle polymer exhibit high toughness and strength, besides, need to use of plasticizer to make them softer to use in biomedical applications [5,7,16].

Applications of PVC in biomedical area is wide, such that, almost 25% of all polymer resin to produce medical devices is PVC [9]. Some of its applications are, blood storage bags, extracorporeal tubings, catheter bottles, surgical packaging, dialysis devices, feeding, tubing [5,7,9,16].

1.3.6 Polyamide

Polyamide (PA), also known as nylon, is a block copolymer with a interchain hydrogen bonding and high crystallinity and high tensile strength [7]. Due to its excellent tensile properties, PA is mostly used for suture materials, ligament and tendon repair materials, balloon of catheters and dialysis membranes [5].

1.3.7 Poly(methyl methacrylate)

Polymethyl methacrylate (PMMA) is a linear chain, amorphous homopolymer with a high rigidity, thoughness and weathering properties, therefore widely used in dentistry and orthopedics, i.e. bone cement [5,7,16]. PMMA have also unique optical properties (Plexiglasss), that 92 % light transmission, and its highly hydrophilic, which allows their use in ophthalmology, as intraocular and hard contact lenses [5,7,9,16].

PMMA is broadly used in the biomedical beside mentioned above, as blood pump and reservoirs, blood handling components, catheter, urological accessories, chest drainage units [5,7,9,16].

1.3.8 Poly(tetrafluoroethylene)

Polytetrafluoroethylene, also known as Teflon, is a homopolymer which have highly thermal and chemical stability [16]. Their chemical structure is $(C_2F_4)_n$ similar to PE, except hydrogen atom, replaced by fluorine atoms. It is highly hydrophobic and has great lubricity. It has expanded, microporous form (ePTFE), which is commercially called as Gore-Tex, used mainly as vascular grafts, catheters, patches for hernia repair and surgical sutures in biomedical area [5,7,16].

1.3.9 Poly(dimethylsiloxane)

Polydimethylsiloxane (PDMS) is a homopolymer which has silicon-oxygen backbone replaced instead of carbon backbone in its chemical structure [16]. PDMS have excellent stability and flexibility and highly inert polymer [5,16]. PDMS is used in biomedical applications as blood vessels, heart valves, dialysis membranes, catheters, drainage tubing, finger joints, chin and nose implants [5,16].

1.3.10 Poly(carbonate)

Polycarbonate (PC) is a type of polyester, with excellent mechanical and thermal properties, impact resistance, hydrophobicity and antioxidative properties [7,9]. PC is amorphous and transparent polymer, used in biomedical area as high pressure syringes, artery cannulas, insulin pens, glucose meters, luers, stopcocks, suture materials, dialysis membrane and containers [5,9].

1.3.11 Poly(ethylene terephthalate)

Polyethylene terephthalate (PET) is a polyester, commercially called as Dacron, with a unique chemical and physical properties due to its highly crystalline structure and hydrophobicity [5,7,13]. PET is widely used in biomedical area as ligament and tendon repair materials, sutures, surgical meshes, vascular grafts, heart valves [5,7,13].

1.3.12 Polyether sulfone

Polyether sulfone (PES) is a type of polyester, with amorphous structure and have excellent thermal performance and optical clarity [9]. Due to its thermal properties and amorphous structure, its mold shrinkage is low, therefore good candidate for applications which require small tolerances to dimensional change. PES used in biomedical area as dialysis membranes, fluid handling couplings and fittings, medical devices which needs repeated sterilization [5,9].

1.3.13 Polyether ether ketone

Polyether ether ketone (PEEK), also known as polyarylketone, is a polyether with extraordinary mechanical properties, that Young's modulus of 3.6 GPa and tensile strength of 170 MPa [9]. PEEK have high resistance to thermal degradation, high chemical resistance and superior wear resistance, therefore it is so suitable material for orthopedic load bearing applications. Their applications in biomedical area are, orthopedic implant parts, inner lining of catheters, keyhole surgery devices, disposable surgical instruments, dental syringes [5,9].

1.4 Biocompatibility of polymeric materials

Biocompatibility of the polymeric biomaterials can be considered mostly by means of its bulk and surface properties. Mechanical compatibility is important for sufficient stability and appropriate rigidity within bulk properties, whereas material surface compatibility is important to obtain a good interfacial biocompatibility. Besides, chemical compatibility is also equally important.

When a biomaterial is placed into the body, firstly its surface comes into contact with physiological fluids, thus first interaction is strongly dependent on the surface properties, which means biological interactions mostly related to biomaterials surface therefore surface properties play a key role on biological response of the living systems. Principally, non-toxicity is one of the required feature for prospective implants, also non-allergic response, noncarcinogenesis and nonpyrogenecity must be take into consideration [7]. Sterilization ability is important to enhance surface biocompatibility.

Most of the polymeric surfaces are open for bacterial adhesion due to their lack of antibacterial surface compatibility, therefore infections stemming from microbial interactions (especially, nosocomial infections during hospitalization) are unavoidable [19,20]. This is a big drawback which may cause patient discomfort, re-operation and external antibiotic drug load, furthermore, loss of viability of the related tissue or even death of the patient.

Another disadvantage of the polymeric biomaterials, especially used in tissue engineering, is insufficient cellular interactions in terms of cell adhesion and proliferation, therefore, inactive surface of the polymeric biomaterial do not react with the living tissue and may cause foreign body reaction or extent the healing time of the diseases.

In the case of using a polymeric biomaterials as a blood contacting device, it may cause surface induced thrombosis since its surface is not active as living body [21,22]. Resultant thrombus may cause vascular occlusion (blocking the blood flow by blood clot), which results in serious health problems [23,24].

So that, superior mechanical, chemical and monetary advantages of polymeric biomaterials undergoes undesired for mentioned applications because of their insufficient surface interactions with the living body, environment and blood cells. In general, surface properties of polymeric biomaterials described with surface wettability, surface chemistry, surface roughness and surface charge [25]. Therefore, change in surface properties directly influence the bacteria and cell adhesion/proliferation, blood protein adsorption and thrombus formation.

Surface treatment and/or modification of polymeric biomaterials can provide reactive surface properties to the polymer to deal with bacterial adhesion, enhancing cell interactions and avoid biomaterial induced blood thrombosis. Thus, beside its bulk material advantages, thanks to availability of creating bioactive surfaces can provide them significantly improved biocompatibility by changing their physico-chemical structure.

1.5 Surface modification of polymeric materials

Surface modification of polymers is beneficial for changing the surface characteristics to minimize the microbial attachment to avoid microbial infections and maximize the cell adhesion and proliferation ability to speed up the tissue regeneration and protein adhesion level to enhance hemocompatibility. Surface modifications changes polymer's surface energy, polarity, topology and chemical composition, therefore changes its interaction with the environment and living tissue, nevertheless, without changing its bulk properties during surface modifications must take into consideration to do not lose its desired bulk properties.

Surface modification of polymeric biomaterials by using chemical agents (both synthetic and natural materials) is promising approach for enhanced surface interactions with the living tissue according to agent's specific properties. There are several methods to modify a polymer surface: wet-chemistry, ozone induced treatment, corona discharge, UV irradiation, flame treatment and plasma treatment [26-29]. It is necessary to take into account the heat range during the treatment process to keep the advantageous of polymer's bulk properties and toxic residues content minimization after the treatment. Moreover, expense of used chemicals and technology is also important for industrial applications.

Most of the polymers, especially polyolefins (such as polyethylene, polypropylene), are very inert materials with a hydrophobic surface character. Therefore, their surface modification with another chemicals is their biggest drawback. Increasing the surface hydrophilicity and functionality is needed before such immobilizations. Plasma treatment can provide favorable surface conditions by tailoring the surface without needs of chemicals in seconds. On the other hand, plasma treatment is heat free process and its interaction is limited with top layer of the surface up to 1 μm [30-32], therefore it is possible to keep bulk properties of the polymer during the process, which is explained in detail in next chapter.

1.6 Plasma surface treatment

Plasma was defined as the fourth state of the matter by Sir William Crookes in 1878 and named as plasma by Irving Langmuir in 1928 [33]. Even if plasma is the latest defined state of the matter, more than 99% of the universe is in the plasma state, i.e. sun, stars, stellar interiors, atmospheres, gaseous nebulae, solar winds, lightning, etc. [34,35]. Plasmas, which in use in other 1%, are produce in laboratory conditions.

Plasma is electrically neutral ionized gas produced by applied energy, by means of heat or electrical field to a gas to excite its atoms to break apart into ions and electrons, which called ionization of gas (Fig. 1.4). Therefore, plasma is a high energy state of matter and contains excited atoms, electrons, positive ions, neutral species, free radicals and photons [25,33,36-39].

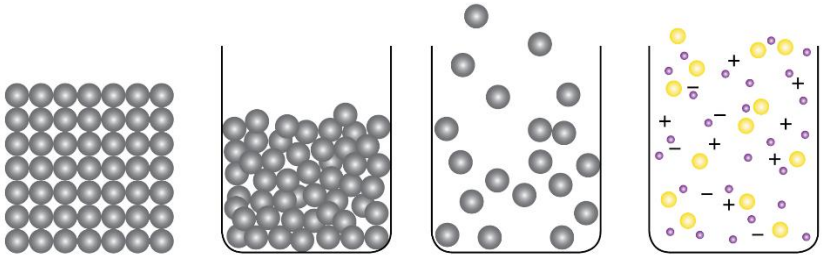

Fig. 1-4: States of matter from left to right: solid, liquid, gas and plasma phase.

Plasma can be classified as thermal and non-thermal plasmas according to the ion and electron temperature [35,40]. Thermal plasmas are in equilibrium state by means of temperatures of the ions (T_i) and electrons (T_e) and the gas (T_g). The thermal range from 4000 to 20000 K according to ionization temperature of the elements ($4000K < Ti = Te = T_g < 20000K$), furthermore, density of ions (n_i) and electrons (n_e) are also equal [35]. Thermal range differs according to ionization temperature of the elements. For instance, while 4000 K is enough to ionize cesium, 20000K needed to ionize helium. It is rather complicated to generate in laboratory scale.

Non-thermal plasmas, also called cold plasmas and/or low temperature plasmas, are non-equilibrium by means of ion, electron and the gas temperature. Most of the laboratory scale plasmas are cold plasmas generated by electrical discharges. Ion and gas temperature of the cold plasmas is around the room temperature of 300 K while electron temperature is around 20000-50000 K due to applied power heats the electrons. Therefore, applied power heats the electrons up to few thousands Kelvin while ion and the gas temperatures are around the room temperature of 300 K.

Most of the laboratory scale applications made by cold plasmas. Cold plasmas can be produced under atmospheric pressure and/or low pressure, called as atmospheric pressure plasmas and vacuum plasmas, respectively.

Different kinds of atmospheric pressure plasmas can be produced by high electrical field exposure under atmospheric pressure (~760 Torr = 10^5 Pa = 1 bar), such as corona discharge, arc discharge, dielectric barrier discharge (DBD), atmospheric pressure glow discharge (APGD), plasma jets, etc. [41]. The biggest advantage of atmospheric pressure plasma is technical compatibility that rather cheap and easy to produce without vacuum components.

Low pressure plasmas produces in a vacuum chamber. Pressure range is between 10 mTorr and 10 Torr and applied voltage between anode and cathode is a few hundred volts [35]. According to applied current, low pressure plasmas can be divided to two 2 types : Direct current (DC) plasmas and alternative current (AC) plasmas. DC plasmas is also called as glow discharge, which can produce by low frequencies (kHz range). Radio frequency (RF) plasmas, microwave (MW) plasmas are belongs to AC plasmas, which are generated by higher frequencies (13.56 MHz and 2.45 GHz, respectively).

Carrier gas to produce plasma is vary depends on the application. Cleaning and sputtering process often carried out by using argon, helium and neon gases due to due to their inert nature, which do not take place chemical reaction with the applied surface. Deposition and implantation processes often use argon, xenon and krypton. Enhancing surface adherence and printability by increasing wettability may require reactive gas, such as nitrogen [42,43]. Increasing the surface energy by etching and oxidative functional groups incorporation, oxygen plasmas are often used [44,45]. Surface etching might be performed by fluorine containing plasmas and increases the surface hydrophobicity, as hydrogen containing plasmas [46]. Therefore, plasmas generated with oxygen used as a carrier gas probably the best choice for polymeric materials to enhance surface energy for further modifications due to its dual advantage of surface etching and surface functionalization by oxygen containing groups.

Thereby, influence of the plasma on the material surface is depends on discharge parameters, i.e., carrier gas and its flow rate, applied voltage, frequency which creates different plasma type and/or differs plasma properties, i.e., ion and electron density and energy of the plasma.

Surface modification by plasma treatment is common and efficient way to enhance surface compatibility of the metals, polymers, textile products. Especially, non-polar materials, such as polymers have low surface energy which makes them challenging materials for surface modification. Plasma treatment of polymers is widely used process to tailor physical and chemical properties of their

surface to enhance its wettability, surface energy, adhesion ability, etc. It is generally used for etching (ablation), activation and crosslinking [47,48].

Etching of the surface by plasma species is a sputtering process to removing materials and/or contaminations from the surface in chemical and/or physical ways, and tailoring the morphology by increasing the surface roughness (Fig. 1.5). Etching may cause the polymer degradation by breaking the covalent bonds of polymer backbone [47]. Therefore, processing parameters, such as time, density of active plasma species, power, frequency and applied gas are determines the etching degree [36,48].

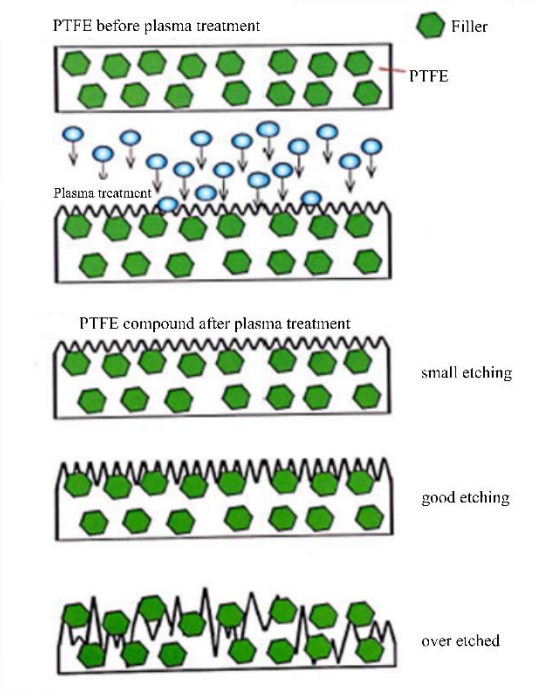

Fig. 1-5: Schematic illustration of plasma etching on PTFE [Diener.com].

Activation of the polymer surface leading to create free radicals by high energy of UV radiation of plasma [47] and separation of hydrogen from polymer backbone [48]. Therefore, existence of functional groups of polymer surface increase and become more capable to carry out further chemical reactions (Fig. 1.6) This process also called as functionalization. Due to the initially existed reactive groups in some of the polymers and/or ease of incorporation of reactive functional groups by plasma treatment make them promising materials among biomaterials [5].

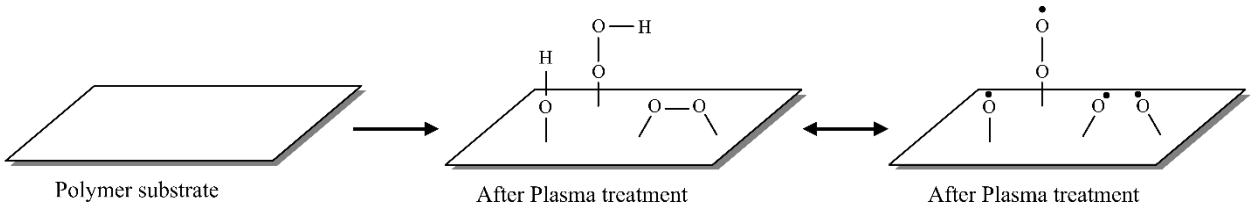

Fig. 1-6: Schematic illustration of a polymer surface, before and after plasma treatment.

Plasma processing with an inert gas results in expel hydrogen atoms from the polymer backbone and produce a crosslinked surface. Therefore, polymer surface is hardened by this phenomenon called as CASING (crosslinking by activated species of inert gases) [41,48,49]. Except hardening the surface, crosslinked surface layer is suitable for adhesive bonding [50,51]. It has been demonstrated that UV radiation induce crosslinking via C-C and peroxy (-O-O-) linking of macromolecules [41].

Modified thickness of the polymer surface depends on the processed polymer type, type of the plasma reactor and parameters (power, frequency, wavelength, used gas, processing time, etc.), nevertheless, it is in the nanoscale [41,48]. Thus, bulk properties of the materials expose to plasma remain unchanged. There are many advantages of plasma process on surface modification of polymeric materials, beside some of the minor disadvantages, listed in Table 1.2.

Table 1.2 Some of the advantages and disadvantages of the plasma treatment.

Advantages	Disadvantages
Environmentally friendly (Solvent free process)	Expensive tools and setup
Fast process (In seconds)	Reason of chemical interactions occurred by plasma are not so clear
Pinhole free films	Difficulties of the control the process since it is so fast
Uniform thickness (proportional to time)	
Homogenous treatment	
Heat free process (no damage on substrate)	
Influence of plasma is limited to surface, does not affect the bulk properties	
Can be applied any surfaces	
No sample preparation before application	

1.7 Bioactive surface coatings of the polymeric materials

Due to the fact that synthetic polymeric materials are not adequately biologically active, therefore their interaction with the biological environment is limited and challenging to adapt them to living tissues. Recent years, increasing biocompatibility of polymeric biomaterials is an attractive topic since their enormous use in biomedical applications, food and pharmaceutical packaging, drug delivery systems. Therefore, modulating biological response of the surrounding biological environment by designing bioactive systems is promising. Bioactive polymer systems are able to modulate interaction between the material and contacted living tissue, such as increasing cell adhesion and proliferation performance, avoid bacterial adhesion and colonization which may cause serious infections and bring in antithrombotic properties to avoid biomaterial induced blood thrombosis, in the case of their use in blood contacting devices.

Bioactive polymer systems basically consists of bulk polymeric material incorporated with bioactive agents (BA) which have specific bioactive effects (Fig.1.7). Bioactive polymer systems are divided to two according to the releasing mechanism of the used bioactive agent, as migratory and non-migratory. In the case of migratory bioactive polymer systems, bioactive agent release to the surrounding environment (either volatile or by contacting) due to its special type of immobilization method onto the polymeric material. In the case of non-migratory bioactive polymer systems, bioactive agent is stable in the matrix and can not release due to its strong immobilization by covalent bonding. For polymeric materials used as biomaterial, both migratory and non-migratory bioactive coatings are preferable, i.e. for wound dressing, migratory coating might be better to penetrate the injured tissue, however, their use in implantation, non-migratory coating is more desired to keep the chemical agent onto the surface which is not needed to penetrate into the living tissue.

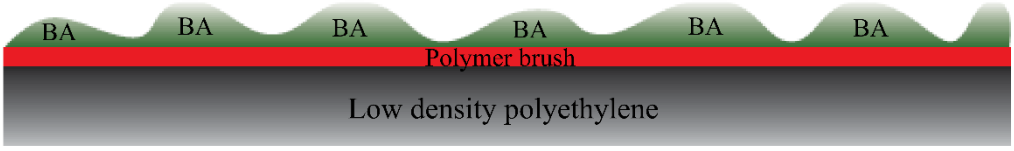

Fig. 1-7: Schematic diagram of a bioactive surface.

There are two general aspects to create bioactive polymer systems: First, producing polymer blends with special bioactive agents to obtain bioactive polymeric material. However, creating these systems are costly and most of the cases only the surface of the material is important to be bioactive instead of whole substrate due to contact with the surrounding tissue is limited by the surface. Second approach is bioactive surface coating of synthetic polymeric materials,

which become promising approach to reduce using of bioactive agents, and cost of blending, moreover, producing of more homogenous bioactive surface consist of only the desired biomolecules. Therefore, drawbacks of bulk polymer properties on contacted living tissue can be eliminated by bioactive coatings.

However, hydrophilic nature of polymeric materials makes them challenging to immobilize bioactive agents. Thus, their hydrophilicity and surface energy must be increased as it discussed in section 1.6, in detail. In addition, increasing the chemical bonding performance of bioactive agents, creating polymer brushes of amine group containing monomers onto the functionalized polymer (i.e., by plasma treatment) is promising approach. Consequently, bioactive polymer system can be produced by such multi-step approach, which is explained in section 2, in detail.

1.7.1 Bioactive surface coating to avoid biomaterial induced infections

Besides adequate physical and chemical properties, one of the biggest drawback of the biomaterials, including metallic, ceramic and polymeric biomaterials, is vulnerability of bacterial contamination during the hospitalization or implantation [52,53]. Such postoperative bacterial contaminations are stemming from either endogenous (internal) and/or exogenous (external) sources and may cause serious infections, which threats patient's healt, prolonged hospitalization or healing time, additional drug load, require of replace the infected biomaterial by secondary surgial operetation, lead to excessive cost and even may cause patient's death [54-63]. Furthermore, bacterial contamination is a big threat for medical devices and tools used in surgery during the hospitalization period.

Nosocomial infection (nosocomium in Latin, which means hospital) is one of the most important infection hospital acquired infection, derive from hospital equipments, medical tools, other patients, environment (air, food, beverages, temperature, pH) and implanted biomaterials or even by existence of pathogens inside of the patient's body. Such that, It has been reported approximately 1.7 million healt-care associated infections, (such as, blood stream infections, pneumonia and urinary tract infections) only in USA and 100.000 of them are lead to dead [64]. Hygiene of the hospital environment, medical devices and tools has importance, however resistance of biomaterials to bacterial colonization is equally important to avoid nosocomial infections.

Nosocomial infection, as most of the other infections, influenced by patient conditions, such as immune system, age, sex, diseases and usage of other medical supplements. Type and level of pathogens and their behavior under certain circumstances, such as environmental conditions, patient's immune system reactions.

Adhesion ability of bacterial strains onto the biomaterials implanted to the body, such as catheters, sutures, artificial joints, dental and orthopedic implants, vascular grafts, heart valves, fixation devices, is another important criteria to determine the level of infection. Therefore, it depends on biomaterial's physical and chemical surface properties, topography, surface hydrophilicity, surface charge and density [54,64,65].

In brief, nosocomial infection depend on environmental conditions, type of bacterial strain, patient status and surface condition with its bacterial interaction properties of the biomaterials.

Bacterial contamination of biomaterials surface is mediated by physico-chemical interactions between bacterial strain and the biomaterial surface by means of gravitation force, van der Waals force, electrostatic force due to charge density, chemotaxis, galvanotaxis and haptotaxis [16,54,65], followed by adhesion of the bacteria onto the surface by means of hydrogen bonds, and bacterial structures such as pili and fimbriare. This attachment occurs rapidly and followed by cellular proliferation by multilayering the cells and clustering. Composed bacterial cells secretes extracellular matrix (mainly consist of polysaccharides, nucleic acids and proteins) to cover the colonies to create biofilms, as depicted in Fig. 1.8 [66]. Reaching the critical amount of bacterial strain in the colonies cause rupture of the biofilm, and releasing the bacteria to the surrounding tissue, which may cause serious infections. Such inflectional process takes about three months after the implantation and it is challenging to remove existed biofilm, moreover it is a potential source of further infections [60,66]. It need use of antibiotics for long time to heal, even secondary surgery to replace the contaminated biomaterial with the new one, which cause patient's discomfort and side effect of drug load, also lead to additional cost.

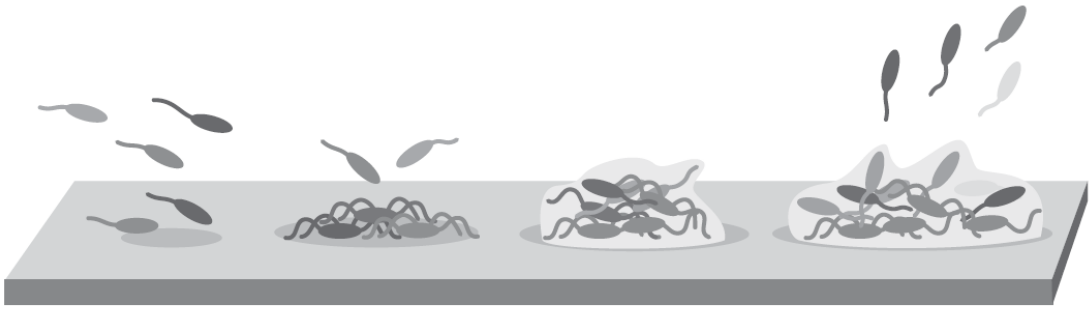

Fig. 1-8: Schematic illustration of biofilm formation followed by bacterial releasing.

There are both gram positive and negative bacterial strains responsible for nosocomial infection, such as *Staphylococcus aureus,* methicillin resistant *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis, Klebsiella pneumoniae, Mycobacterium tuberculosis, Mycobacterium avium-intracellulare, Enterobacter aerogenes,* coagulase-negative staphylococci, *Escherichia coli* and so on [66,67]. Table 1.3 shows some of the bacterial strains with their infection rates according to used biomedical materials, which indicates application type and places are also play an important role on the level of infection, which is mostly owing to being lack of protection of the body without unbound, such as skin or scab (i.e., in dental, venous and urinary regions).

Table 1.3. Bacterial strains with their infection rates according to used biomedical materials [66].

Biomaterials	**Pathogens**	**Infection rate**
Central venus catheters	*Staphylococci, Klebsiella species, Candida albicans, etc.*	10 – 40 %
Urinary catheters	*Candida, Klebsiella pneumoniae, Escherichia coli, Pseudomonas species, etc.*	10 – 30 %
Fracture fixation device	*Staphylococcus aureus, coagulase-negative staphylococci, Gram-negative rods, etc.*	10 – 30 %
Dental implants	*Porphyromonas gingivalis, Enteric and Candida species, etc.*	5 – 10 %
Spinal	*Staphylococcus aureus, Mycobacterium tuberculosis, Gram-negative rods, etc.*	2 – 2.76 %
Knee	*Staphylococcus species, Streptococcus pneumonia, etc.*	0.4 – 5 %
Hip	*Staphylococcus aureus, Cryptococcus neoformans,etc.*	0.23 – 2.33 %
Shoulder	*Staphylococcus species, Mycobacterium aviumintracellulare, Propionibacterium acnes, etc.*	0.04 – 4.4 %

Staphylococcus species, especially *Staphylococcus aureus* is one of the common gram positive round shaped coccal anaerobic pathogen (Fig. 1.9) which promote such biomaterial induced inflammation by means of inactivating antibodies by binding of produced protein toxins. Due to its enzymes and cytotoxins secretion, *S. aureus* can easily proliferate on living tissue. Its resistance to antibiotics is one of the biggest problem due to creating methicillin resistant *Staphylococcus aureus* therefore challenging to get rid of it by antibacterial agents.

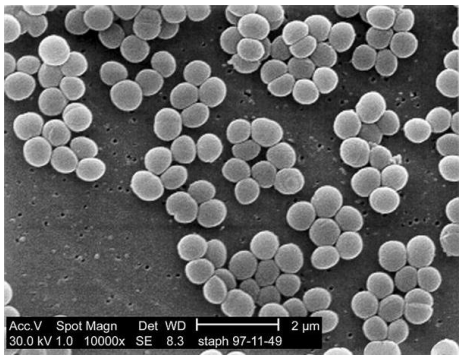

Fig. 1-9: S. aureus strains [brighthub.com]

Escherichia coli is another most common gram negative rod shaped facultative anaerobic pathogen (Fig. 1.10) which cause various infections. Due to their ability to existence even out of the living body, and fast reproduce time (about 20 minutes) makes them potential pathogens for bacterial contamination.

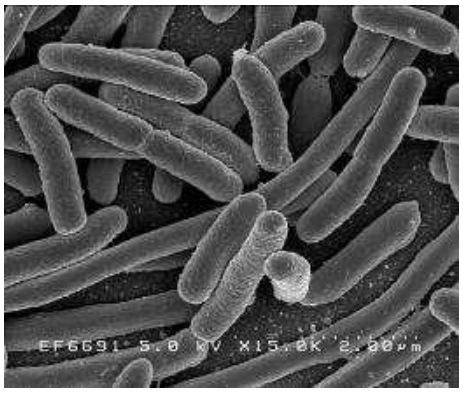

Fig. 1-10: E. coli strains

Thus, *S. aureus and E. coli* are commonly used strains representative of gram positive and gram negative bacteria for antibacterial researches.

Antibacterial agents used to treat such infections works as bacteriostatic (biocide) with an aim of inhibiting bacterial growth, and bactericidal with a purpose of bacterial death [68]. They can be used in oral, parenteral or local way, and its dosage with duration set according to situation of the disease or condition of the patient. However, there is no precise way to design certain dosage and period of use, therefore it may even extent the healing time or increase the side effect because of redundant drug load. There are enormous antibacterial agents to use in medicine, which are continually developing with a target of obtaining the optimal healing time, extended efficient onto the bacterial strains and straggling with the increasing resistance of the bacterial strains against antibacterial agents.

Chitosan is one of the most used natural polymer derived from animal source which show initially antibacterial effect [69,70]. Chlorhexidine is another effectively used antibiotic with a broad spectrum of gram positive and negative bacterial strains, however it has been found that it is cytotoxic for periodontal tissue, fibroblast cells, kidney cells and may threat health due to its degradation products.[71,72].

Triclosan is another antibacterial agent, with a broad spectrum and chemical stability, used to treat infections but some of the bacterial strains has resistive to it and its degradation products may threat health.

Fluoroquinolones (FQs) have broad effect against both gram positive and gram negative bacterias antibiotics to treat various infections in dermatology, ophthalmology, and urinary systems [73] by penetrating their cell walls to inhibit DNA gyrase with tolerable side effects [73,74]. There are various derivates of FQs which were produced to treat various infections.

Sparfloxacin (SpF) is a newer third generation fluoroquinolone derivative (Fig.1.11) mainly used for treatment of urinary tract infections, bacterial conjuctivitis, chronic bronchits by inhibiting topoisomerase II (DNA gyrase) and topoisomerase IV [73,75-79].

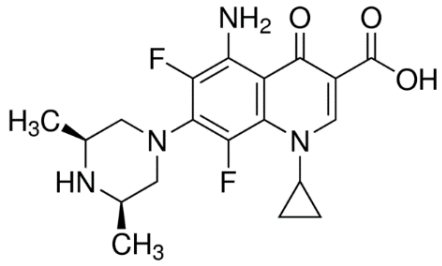

Fig. 1-11: Chemical structure of sparfloxacin.

Enrofloxacin (EnF) is the first generated fluoroquinolone derivative (Fig.1.12), especially developed for veterinary use to treat urinary, respiratory, skin and tissue tract infections by inhibiting bacterial DNA replication and transcription [80-83].

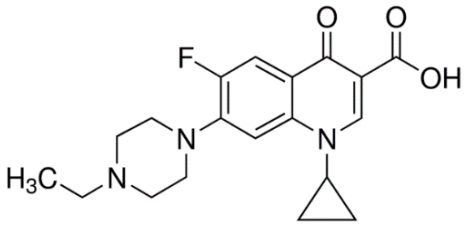

Fig. 1-12: Chemical structure of enrofloxacin.

Lomefloxacin (LmF) is a second generation of FQs (Fig. 1.13) with an antibacterial activity against gram positive and negative bacteria strains to treat soft tissue, gynecological, ophthalmological infections [84].

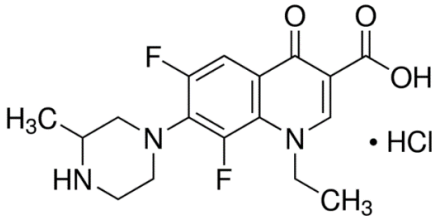

Fig. 1-13: chemical structure of lomefloxacin.

Even if usage of such antibacterial agents are extensively used to treat bacterial infections, there are also various of drawbacks, such as creating resistive pathogens againgsantibiotics (drug resistance bacteria, i.e., MRSA, drug resistant gram negative basili, *E. coli, Pseudomonas aeruginosa*), therefore, prolonged hospitalizaiton, increase the need of medical treatments, external cost and increased mortality [66].

Avoiding biomaterial induced infection is possible by reducing or hindering the bacterial adhesion onto the surface in advance, therefore inhibit biofilm formation with its threatenings. Due to the fact that polymeric biomaterials are vulnarable to bacterial adhesion, and their extensively usage in biomedical applications thanks to their unique properties with industrial and commercial benefits, their interaction wiht the bacterial strains is focus of interest in biomaterial research area.

One of the most used approach to lowering the antibacterial contamination of polymeric biomaterials is blending selected sythetic polymers with the antibacterial agents (both natural and/or sythetic ones) or copolimerization of antibacterial agents with monomer to bring in antibacterial properties [53]. However, by this way, usage of the antibacterial agents is relatively more, in parallel with amount of the used synthetic bulk polymer. Moreover, existance of such agents cause drastically changes in adequate physical and chemical properties of the bulk polymer.

Due to the fact that, only the surface of the implanted biomaterials have contact with the surrounding tissue, tailoring only their surface to be more resistive to bacterial adhesion can bring in various advantages, such as majorly lowering the antibacterial agent usage, therefore lowering the cost, eliminate labour and cost of blending process, and keeping desired bulk properties of the polymer intact.

Surface coating can be achieved by direct incorporation of antibacterial agent by immersing to its solutions but most of the polymeric surfaces, especially for polyolefins (such as polyethylene, polypropylene), is challenging materials due to its highly hydrophobic surface characteristics and low surface energy value. Thus, treatment of the surface for further antibacteiral agent modifications is required [85,86]. There are several methods to treat surface, such as wet chemistry, acid etching, UV irradiation, corona discharge, flame treatment and plasma treatment. Points to take into consideration beside keeping bulk properties intact, are avoiding toxic residues contamination and homogenously treating the entire surface. Even if surface coating of the material is local application, immobilization of the antibacterial agent must be strong to lower the contamination of surrounded tissue by spread agent.

Plasma treatment is unique method to treat polymer surface to increase its hydrophilicity since its nonthermal, fast and homogenous process for surface etching. Moreover, it is able to create oxygen containing functional groups on the surface (such as hydroxyl, carboxyl and carbonyl) by interaction with excited atoms, neutral species and ultraviolet light, which are desired for further chemical reaction with monomer, followed by antibacterial agent immobilization [10,53,87]. In this way, the content of antibacterial agents is minimized and due to their strong covalent immobilization with introduced functional groups by plasma treatment, bioactive surface become more stable and their release to the human body can be controlled. Moreover, exposed polymer surface to plasma can be sterilized without needs of any other chemical by means of bacterial death by destroying their cell wall, thus, eliminate initially existed contamination can be eliminate before immobilize the antibacterial agents to create bioactive surface.

Ability of creating optimal bioactive surface, therefore, depends on plasma type and its processing parameters (voltage, frequency, exposure time, used gas and its flow rate, etc), environmental factors (temperature, pH, moisture), selection of adequate antibacterial agent agains to the certain bacterial strains and its toxiciticiy, bulk properties of the polymer. Morevoer, feasibility of technical needs and economical point of view must be take into consideration.

On the other hand, plasma parameters and process must be controlled to do not damage polymeric surface by exposing the plasma longer than 20 minutes or by using higher power condition for polymeric materials since they are more sensitive than metallic, ceramic counterparts.

1.7.2 Bioactive surface coating to enhance cell interaction

As mentioned, owing to sythetic polymeric materials are biologically inert, beside bacterial infection risk, another drawback is insufficient cellular interactions, in terms of cell adhesion and proliferation which need to be promoted to improve cellular interactions to accelerate the tissue regeneration.

Tissue engineering deals with enhance cell adhesion and proliferation and matrix formation [39]. Design of polymeric scaffolds (artificial extracellular matrix) to formation of three dimensional structure as a new tissue is the common way to promote cell adhesion/differentiation [88,89]. Synthetic polymers used in tissue engineering can be also prepared by surface modification to create bioactive polymer systems with thin film coating of eligible chemical agents to recruit cellular interactions accelerate wound healing and tissue growth. As such in surface-bacteria relationship, cellular interaction is also associated with surface properties, such as topography, hydrophilicity, surface chemistry and charge, etc.

Cell adhesion and proliferation is enormously depends on protein adsorption onto the polymeric material by means of integrin receptors responsible for cell adhesion interactions, therefore, modulating the surface-protein affiliation is important to facilitate cell growth [90,91].

In the clinical tissue engineering, fibroblast cells (Fig.1.14), which found in connective tissues, are responsible for wound healing and regeneration of the injured tissue [88,89]. Therefore, fibroblast cell – polymeric biomaterial interaction play a key role for the regeneration of the injured tissue in the living body.

Therefore, creating a bioactive polymer surface to increase adhesion ability of fibroblast cells and facilitate their long term growth is promising approach in tissue engineering.

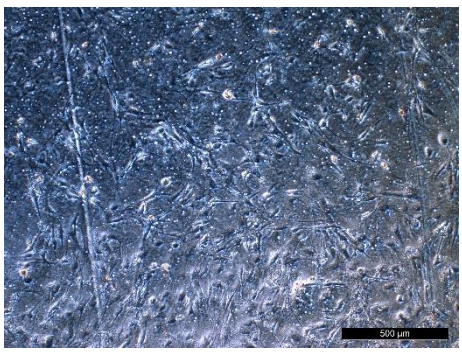

Fig. 1-14: Mouse primary fibroblast cells on low density polyethylene

Polysaccharides are favorable candidates as a bioactive agent to use in bioactive polymer system due to their excellent biocompatibility, superior cellular adhesion and proliferation [92,93]. Chondoitin sulfate (ChS) is one of the most important natural polysaccharide (Fig. 1.15) mainly found in connective cartilage tissue and also in other sources as a extracellular matrix which has an important role on cell functions [93-97]. It is a linear, polydisperse, sulfated polysaccharide which belongs to glycosaminoglycans (GAGs) family [98-100]. It has highly negative polarity due to SO_4^{-2} and COO^- presence [97] which plays significant role on interaction with other constituents by means of repulsive and attractive forces. ChS has very complex heterogeneous structure [98] and occurs in several forms, i.e., Chondroitin 4-sulfate (ChS A), Chondroitin 6-Sulfate (ChS C), dermatan sulfate (ChS B) [94]. ChS is generally produced by extraction and purification from animal tissues [98-100]. ChS has beneficial properties for tissue engineering such as anti-inflammatory effect, wound healing capability and ability to accelerate the regeneration of injured bone [95,96]. It is also used as a dietary supplement for the osteoarthritis treatment [98-100]. Due to the fact that effect of the orally delivered agent is reduced by the digestive system, ChS immobilization onto the polymeric biomaterials (such as surface mediated drug delivery) will have higher concentration, thus, increased effect on the particularly contacted tissue in surgical applications [94].

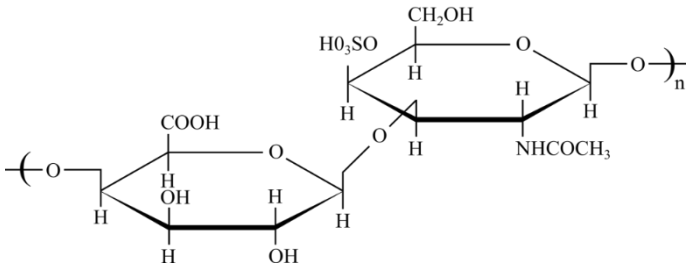

Fig. 1-15: Chemical structure of chondroitin sulfate.

Modification of the polymer surface by plasma, introduces negatively charged functionalities to the surface and further ChS with negative polarity immobilization create electrostatic repulsive force which reduces the binding affinity between them. To avoid this, positively charged mediators, like allyamine (CH2=CH-CH2-NH2) are promising choice to introduce a high density of positively charged amine groups (-NH2) by grafting with a good stability.

1.7.3 Bioactive surface coating to avoid thrombus formation

When is a synthetic biomaterial, with the purpose of implantation or tissue replacement, introduced to a living biological system, firstly its surface comes into contact with blood, and it may cause foreign body reaction and surface induced thrombosis since it is not biologically active as living tissues. First response of the biological system is rapid protein adsorption in accordance with the Vroman Effect [101,102] onto biomaterial's surface in seconds. Therefore it becomes recognizable by the integrin receptors of most of the cells [103]. Thus, cellular interaction with the adsorbed protein layer plays a paramount importance [21,22]. Types, concentration and conformation of the proteins is important by means of further cellular interactions at the interface [102].

In terms of blood compatibility, the blood response strongly depends on materials surface properties as surface chemistry, energy, charge density and wettability [21]. Protein adsorption is followed by platelet adhesion and aggregation, activation of intrinsic pathway of blood coagulation via blood protein factor XII (mostly activated by negatively charged surfaces [91], fibrin network formation, complement system activation with interactions of erythrocytes and leucocytes [22,103-107]. Therefore, this coagulation cascade (given in Fig.1.16) trigger the thrombus (blood clot) formation on the biomaterials surface, namely surface induced thrombosis [21,22], which may cause blood vessel occlusion or heart attack in the case of its vascular implantation [23,24]. Thus, reducing protein adsorption and platelet activity increase the hemocompatibility of the biomaterial.

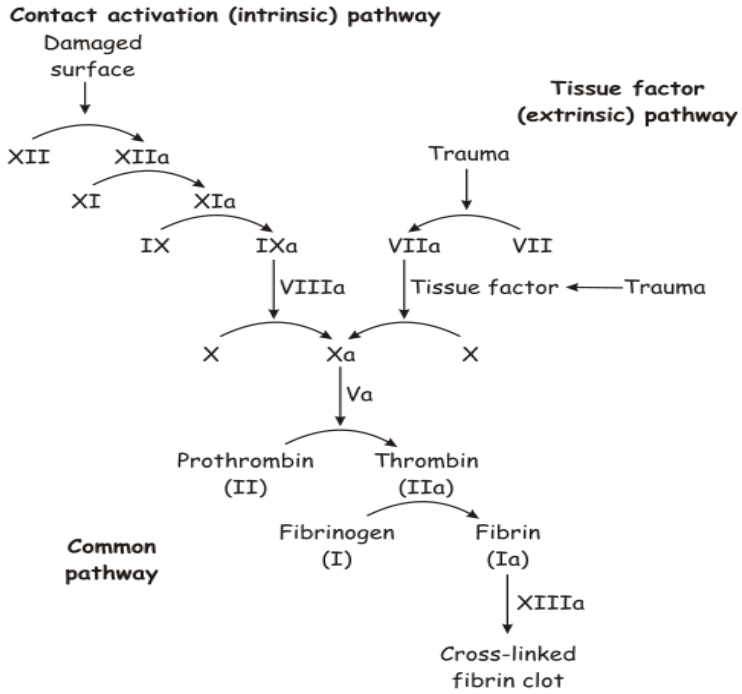

Fig. 1-16: Clotting cascade [mrcpandme.blogspot.com].

To avoid blood coagulation, fibrinolysis occurs by a normal body process [108] as a result of breakdown of blood clots (primary fibrinolysis) or by medical supply (secondary fibrinolysis). Thrombus inhibition by anticoagulant sulfated polysaccharides is promising strategy to avoid thrombus formation. Sulfated polysaccharides are convenient macromolecules for anticoagulation studies. Heparin is a wellknown sulfated polysaccharide (Fig. 1.17) used as a anticoagulant for several years [85,109,110]. Biggest drawback of heparin is hemorrhage and thrombocytopenia [109-111]. It may also cause virus based infections due to the fact that it is mostly obtained from animals [109].

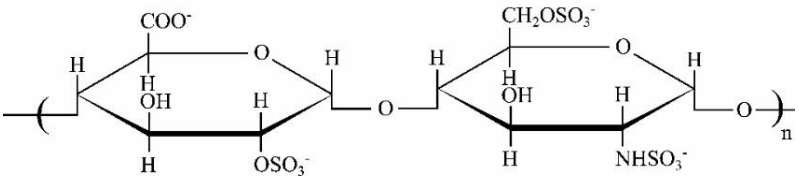

Fig. 1-17: Chemical structure of heparin.

Fucoidan is another sulfated polysaccharide of scientific interest in recent years as an alternative anticoagulant to heparin. Fucoidan is a marine sourced biopolymer enormously found in the intercellular matrix of brown algae [109] and rather limitedly found in marine invertebrates [111-113] (Fig. 1.18). Besides its anticoagulant activity, it has sort of biological activities, such as antivirus, anticancer, antitumor, anti-inflammatory and antioxidant activities. It makes fucoidan an attractive polysaccharide for numerous biomedical application [114-118].

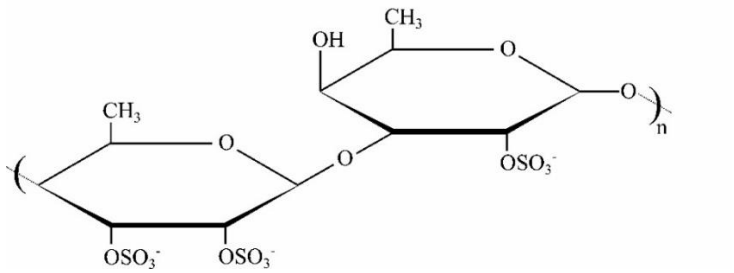

Fig. 1-18: Chemical structure of fucoidan.

Dissimilarly to other polysaccharides, mechanism of anticoagulant activity of fucoidan is related to the interactions with the natural thrombin inhibitors of antithrombin (AT III) and heparin cofactor II (HCII), activated factor II (thrombin) and activated factor X [110,112,119,120]. Effect of the anticoagulant activity of fucoidan depends on its structural properties, such as sulfation pattern and degree, monosaccharide composition and especially its molecular weight (MW) [111,112,119,121]. For instance, low molecular weight (LMW) fucoidan found effective for anti-inflammatory response while middle and high molecular weight fucoidan found more effective on anticoagulant activity related with altering the sulfate groups by changing MW for controlling the binding properties [111,112,117,119]. Besides drawbacks of heparin, it has been found that anticoagulant activity of fucoidan is greater than heparin [122]. On the other hand, structure of the fucoidan is not well defined yet, so then its applications are rather limited presently.

AIMS OF THE WORK

The aim of this study is to develop functional surfaces of a polymeric biomaterial for enhanced its surface interactions with the living systems. Low density polyethylene (LDPE) has been selected as a substrate to challenge with its hydrophobic nature as its biggest drawback for surface modifications and due to the fact that polyehthyle is the most commonly used polymer in industry. Within this scope, multistep physico-chemical approach has been used to modify LDPE surface to bring in active surfaces, as listed below.

- Modifying the surface to obtain increased cell proliferation in the case of its usage in tissue engineering.
- Modifiying the surface to avoid biomaterial induced thrombus formation in the case of using it as blood contacting device.
- Creating antibacterial layer onto the surface to prevent microbial infections.

Regarding the related ideas, LDPE surface treataed by air plasma treatment to increase its surface energy and create oxidative functional groups for further chemical interactions. As second step of multistep physico-chemical approach, amine functional groups were introduced via co-polymerization process of allylamine (AAM), N,N-dimethylallylamine (DAAM) and N-allylmethylamine (MAAM) monomers. For the last step, particularly selected sulfated polysaccharides of chondroitin sulphate (ChS), heparin (HEP) and fucoidan (FU) immobilized onto previously grafted AAM, DAAM, MAAM polymer brushes.
Surface characterization of modified surfaces were carried out by wettability test, AFM, SEM and chemical changes were determined via ATR-FTIR and XPS.
Primary mouse fibroblast cells were used for cell adhesion/proliferation study; human venous blood were used for anticoagulation test; *Staphylococcus aureus* and *Escherichia coli* were selected as gram positive and gram negative bacteria respectively for antibacterial testing.

2. EXPERIMENTAL PART

Preparation of bioactive surface was based on layer by layer coating approach for all three studied surfaces to avoid bacterial adhesion, enhanced cell adhesion and proliferation, avoiding biomaterial induced blood thrombosis. This multi-layer approach consist of three steps: First step is plasma treatment to increasing the hydrophilicity and surface energy by means of etching and introducing oxidative functional groups. Such treated surface, therefore, become more hydrophilic as it easily determined by contact angle measurement (Fig. 2.1).

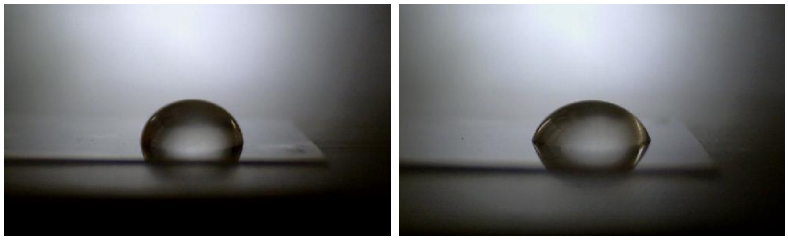

Fig. 2-1: Water contact angle of raw LDPE (83°) and LDPE after RF plasma treatment (68°), respectively.

After plasma treatment of the surface, second step is using amine groups containing monomers to create their polymer brush onto the treated surface is an effective method to increase chemical bonding between the polymer brush and bioactive chemical agent. Allylamine (AAM), N-allylmethylamine (MAAM) and N,N-dimethylallylamine (DAAM) are three monomers with primary, secondary and tertiary amine groups (see Fig. 2.2). Their gas phase exposure to plasma treated polymer surface starts co-polymerization process to create a polymer brush in seconds. Therefore, functionalized surface by this way become more likely to interact further bioactive agent immobilizations.

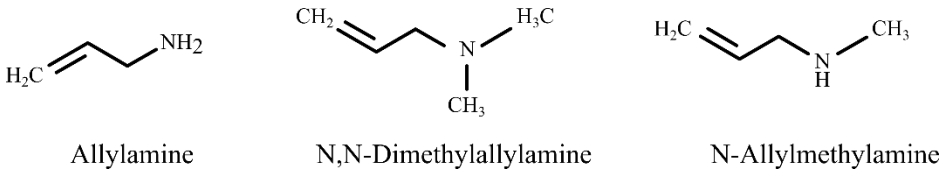

Fig. 2-2: Chemical structures of grafted monomers.

Third step of the multi-layer approach is covalently immobilization of selected chemical agents onto the polymer brushes to create bioactive surfaces specific to increasing biocompatibility.

2.1 Preparation of surface for antibacterial activity

Low density polyethylene (PE) square film with 100 μm thickness and 50 mm × 50 mm dimensions was used as a polymeric substrate. N-allylmethylamine (MAAM) monomer was obtained from Sigma Aldrich (USA) and used as received without further purification. Fluoroquinolones of sparfloxacin and lomefloxacin were purchased from Sigma Aldrich (USA) and enrofloxacin was purchased from Fluka (USA) and their solutions were prepared as 0.5% (w/v) in distilled water at two different pH values of 3 and 6 due to the fact that solubility of FQs varies with pH. The acid pH values were obtained by diluted H_2SO_4 addition into the FQs solutions.

PE substrates were treated by 2.45 GHz microwave plasma, generated by using PICO (Diener, Germany) with an input power of 20 W. Air was used as a discharge gas with 20 sccm flow rate under the chamber pressure of 50 Pa. Process time was set to 60 sec to apply each side of PE substrate and samples hereafter referred as PE_Tre. After plasma treatment, samples were immediately exposed to MAAM vapor atmosphere for 20 seconds to graft polymer brush and named as PE_Tre_MAAM (Fig. 2.3). Finally, samples were placed to solution vials of FQs in pH3 and pH6 separately for 24 hours for immobilization. Prepared final samples labelled as SpF3, EnF3, LmF3 for pH 3 and SpF6, EnF6, LmF6 for pH 6. Finally, the samples were taken out of the solution vials, thoroughly washed by distilled water and dried at laboratory conditions.

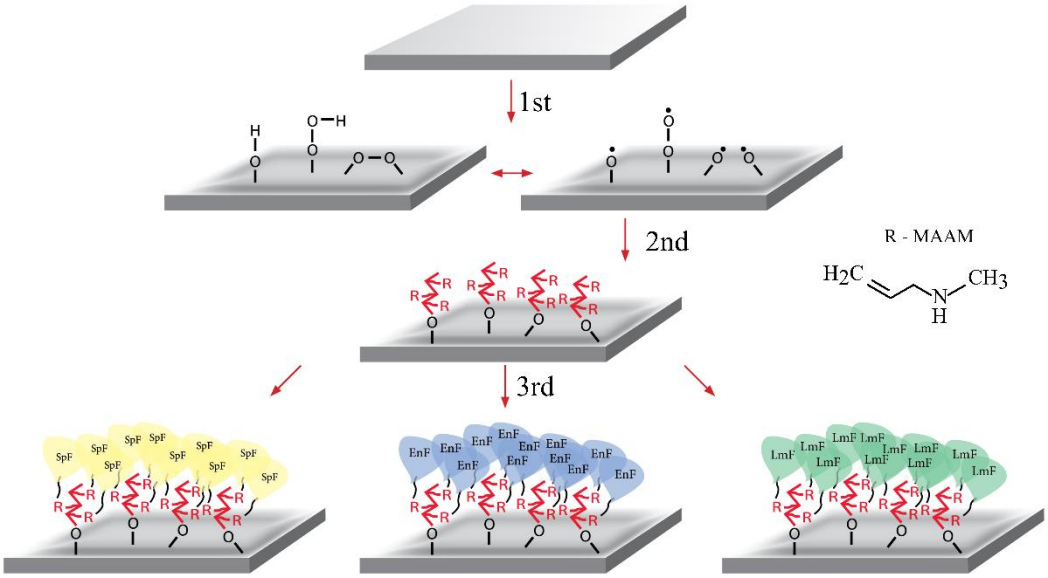

Fig. 2-3: The schematic representation of the process of FQs immobilizations in three steps: First, plasma treatment; second, grafting polymer brushes; third, immobilization of three FQs of SpF, EnF and LmF, respectively.

2.2 Preparation of surface for enhanced cell interaction

Low density Polyethylene (LDPE) film of the 100 µm thickness was obtained and used as received without further purification in the form of square sheets (50 × 50 mm), hereafter referred to as PE. Monomers of Allylamine (AAM), N-allylmethylamine (MAAM), and chondroitin sulfate from bovine trachea (ChS) were obtained from Sigma Aldrich (USA) and N,N-dimethylallylamine (DAAM) was supplied by Fluka (USA). ChS solution has been prepared by dissolving 1% (w/v) of ChS in distilled water. Radio-frequency (RF) plasma was generated by using PICO plasma reactor (Diener, Germany) performed at 13.56 MHz frequency with 50 W of discharge matching power with air as a discharge gas with 20 standard cubic centimeter per minute (sccm) flow rate under 50 Pa chamber pressure. Both sides of each PE samples were exposed to plasma for 60 sec to create free radicals and reactive species on the surface to act as initiator for further copolymerization process and hereafter referred to as PERF. Subsequently, treated PE samples were immediately placed to AAM, MAAM and DAAM vapors for 10 sec in order to immobilize the monomers by radical graft copolymerization process to create functional amine groups containing polymer brushes onto the surface by means of reaction with pre-formed free radicals and samples hereafter referred to as PERFA, PERFM and PERFD, respectively.

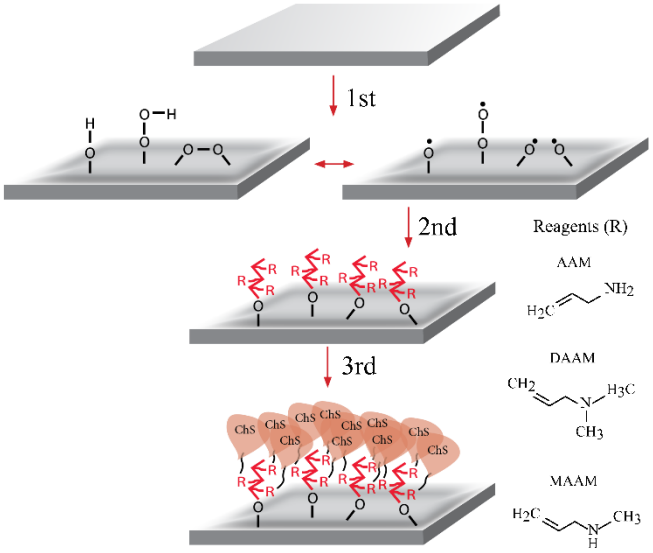

Fig. 2-4: The schematic representation of the process of ChS immobilization in three steps: First, plasma treatment; second, grafting polymer brushes; third, immobilization of ChS.

Each PERFA, PERFM and PERFD sample has been separately placed into ChS solution containing vial for 24 hours at room temperature to immobilize the ChS (Fig. 2.4) to polymer brush of AAM, MAAM and DAAM by means of intramolecular forces. After 24 h of reaction time samples were taken out of the vials and gently dipped into water and then distilled water for cleaning of non-interacted ChS species. Finally, cleaned samples were dried for 2 hours at room temperature. Such prepared samples are labeled as PERFAC, PERFMC and PERFDC according to previously created polymer brushes.

2.3 Preparation of surface to avoid blood thrombus formation

Cylindrical, low density polyethylene (LDPE) Vacuette blood collection tubes (with a 20 cm^2 of contacted surface area) including 4 ml of coagulation sodium citrate 3.2 % was purchased from Greiner Bio-One Company (Austria) to use for anticoagulation activity observation. All blood collection tubes were are thoroughly cleaned with distilled water and dried at 30 °C for 24 h in an oven. LDPE film with the 100 μm thickness was used as a control substrate to LDPE blood collection tubes. The foil was cut into the form of square flat sheets with a dimension of 50 × 50 mm and used as received without further purification. Both sheet and tube form of prepared LDPE samples hereafter referred to as PE. Monomers (see Fig. 5.) of allylamine (AAM), N-allylmethylamine (MAAM) with anticoagulants fucoidan from *Fucus vesiculosus* and heparin sodium salt from porcine intestinal mucosa were purchased from Sigma Aldrich (USA) and N,N-dimethylallylamine (DAAM) was supplied by Fluka (USA). Heparin and fucoidan solutions were prepared as 1 % (w/v) in distilled water and placed to the vials for further use.

Direct current (DC) plasma discharge used for all experiments was generated at the frequency of 40 kHz and power of 50 Watts using a PICO (Diener, Germany) plasma reactor with a volume of 3 dm^3. Air was used as a discharge gas with 20 sccm flow rate and the pressure in the vacuum chamber was 50 Pa. Both sides of each PE sheets and each blood collection tubes exposed to generated non-thermal plasma for duration of 60 seconds (hereafter referred to as PET) to create free radicals and metastable reactive species on the surface to act as initiator for further copolymerization reactions.

After exposing the plasma, samples were taken out of the chamber and immediately subjected to AAM, MAAM and DAAM vapours for 20 sec in order to immobilize them via radical graft polymerization process to create functional amine groups containing polymer brushes onto the surface as shown in Fig. 2.5. These samples are hereafter referred to as PETA, PETM and PETD, respectively. The monomer contact with the radical within its lifetime is of a paramount importance. In our case we expect reaction of monomer with peroxy radicals which have lifetime in the range of a few seconds [123].

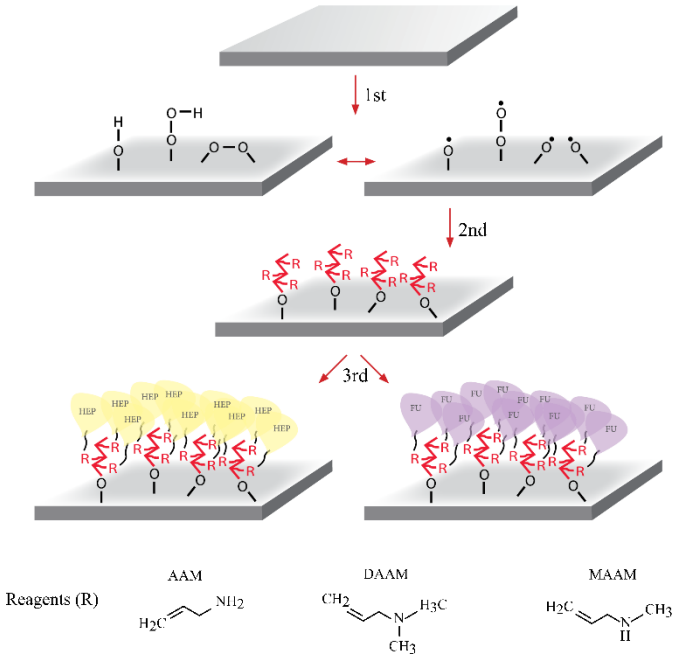

Fig. 2-5: The schematic representation of the process of anticoagulant immobilizations in three steps: First, plasma treatment; second, grafting the polymer brush; third, immobilization of two anticoagulants of HEP and FU, respectively.

Each PETA, PETM and PETD substrate were separately placed into heparin/fucoidan containing solution vials and blood collection tubes has been filled up by the same solution and placed to rotational shaker for 24 hours at room temperature in order to immobilize the anticoagulants to grafted polymer brushes by intramolecular interactions. After 24 h of reaction time, the substrates were taken out of the vials and gently cleaned in water then distilled water to eliminate non-immobilized heparin/fucoidan species. In the case of cylindrical test sample, heparin/fucoidan solutions were poured out and then cleaned carefully by water and distilled water. H and F added to the last place of previous sample abbreviations which are stands for the heparin and fucoidan immobilized conditions, respectively.

Finally, all samples were dried for 2 hours at room temperature. Blood collection tubes were used for anticoagulant activity tests and square flat sheets were used for further tests.

2.4 Characterization techniques

2.4.1 Surface wettability testing

Surface wettability of the materials depends on its surface energy and refers as its hydrophilicity. Contact angle measurement is easy and efficient method to determine the hydrophilicity. Sessile drop method is based on placing a testing liquid, for instance water, onto the surface and measuring spreading water on a surface by means of contact angle as depicted in Fig. 2.6. Measure of the spreading water onto surface represents the water adhesion tension, which estimates the wettability of a surface [22]. Change in tension due to molecular interaction cause change in contact angle in three phase system: solid-liquid-gas interface [124]. Surface which has water contact angle less than 90º defined as hydrophilic and defined as hydrophobic in case its more than 90º. It is due to the amount of hydrogen bond per water molecule that more than one hydrogen bond needed per water molecule to show hydrophilic character [22].

Fig. 2-6: Contact angle diagram.

Sessile drop method was used to evaluate total free surface energy of the samples. We used so called (SEE System) surface energy evaluation system (Advex Instruments, Czech Republic) equipped with a CCD camera. Distilled water was used as testing liquid at 22 °C and 60% relative humidity. The volume of the droplets was 5 μL for each experiment and droplets was kept for 30 s to obtain equilibrium state prior to the measurement. Ten separated readings were averaged to obtain representative contact angle value to estimate surface hydrophilicity.

2.4.2 Surface morphology analysis by scanning electron microscopy

Scanning electron microscope (SEM) is a powerful tool to obtain two dimensional image of the specimen surface with a higher magnification and resolution than that of light microscopes since wavelength of the electron (2.426×10^{-12} m [125]) is much more smaller than wavelength of the visible light ($4\text{-}7 \times 10^{-7}$ m). Therefore, while the light microscope provides a magnification and resolution up to 1500× and 10 microns; SEM is able to reach 60k× and 10 nanometers, respectively [126].

SEM consists of electron source (Tungsten filament, field emission gun or solid state crystal, i.e. LaB_6.), condenser and objective lenses (to produce and electron gun) with apertures and scanning coil (to to scan the electron probe) in a column; sample chamber with a vacuum pump, sample stage, electron detector (secondary electron detector to collect secondary electrons emitted from the specimen or back scattering detector to collect scattered electrons) and visual display monitor (the output signals from the secondary electron detector are amplified and then transferred to the display unit) as shown in Fig. 2.7.

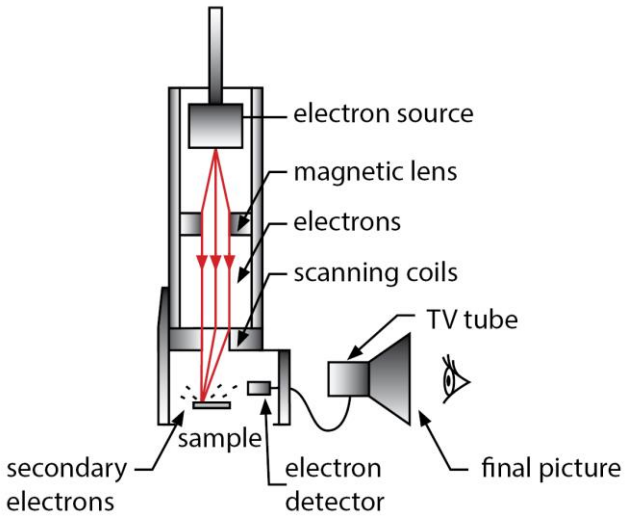

Fig. 2-7: SEM diagram.

Surface morphology of all samples was observed by scanning electron microscope (SEM) using a NANOSEM 450 (FEI, USA) performed at 5 kV. The instrument was equipped with so called low vacuum detector (LVD), therefore further conductive i.e. gold/palladium was not needed, and the measurement was performed at water vapor environment under 90 Pa pressure with a spot size of 50 nm.

2.4.3 Surface topographic analysis by atomic force microscopy

Atomic force microscope (AFM) allows to obtain nano-scale 3-dimensional topography of the surface by scanning. Contrary to other microscopes AFM has no lenses but cantilever with a very thin/sharp tip (Fig. 2.8) and does not required sample preperation.

There are mainly two types of AFM: contact and non-contact AFM. In contact AFM, tip is contacting with the sample surface and features on the surface causes strong repulsive forces against to the cantilever which cause changes in the direction of reflected beams from cantilever and position detector records the reflected beam changes. In the case of non-contact AFM, the tip does not touch the surface and cantilever ossilates above the sample features and same as contact AFM, position detector records the reflected beam from cantilever and create 3-dimesional graph of the scanned surface. Contact AFM may perform by tapping mode as well where the tip is vibrating with a higher amplitude.

AFM works according to the Hooke's law,

$$F = - k . x \qquad (1)$$

where, F is force, k is cantilever spring constant and x is deflection of the cantilever. Cantilever deflection is detect by a laser reflection from cantilever head to a detector. Depends on the changes in the position of cantilever, laser reflection is changes and monitored the topography in this way.

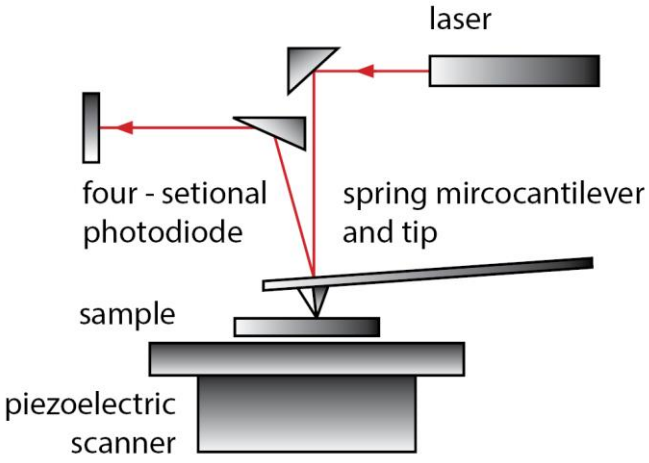

Fig. 2-8: AFM diagram.

Surface topology characteristics on the samples obtained by atomic force microscope (AFM) using a Dimension Icon (Bruker, Germany) with performed by peak force tapping mode with a ScanAsyst-Air Si/Nitride probe (k: 0.4 N/m, Bruker, USA). Scanning area of 5 × 5 μm for each sample was investigated with a frequency of 1 Hz. Average surface roughness (R_a) values were analysed by using NanoScope Analysis software.

2.4.4 Surface spectroscopic analysis by Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy (FTIR) is a vibrational spectroscopic technique for chemical analysis to identify functional groups. It is based on vibration, stretching, bending and contracting of molecular bonds due to absorption of applied infrared radiation by molecules in a particular wavenumber (often given by corresponding frequency) of infrared region [39]. Infrared (IR) region devided to three according to its frequency range: Far-IR in a range of 400 – 10 cm^{-1}, mid-IR in a range of 4000 – 400 cm^{-1} and near-IR in a range of 14.290 – 4000 cm^{-1}, and mid-IR is the most commonly used region in chemical analysis since most of the molecules depicts primary molecular vibration in this region [127].

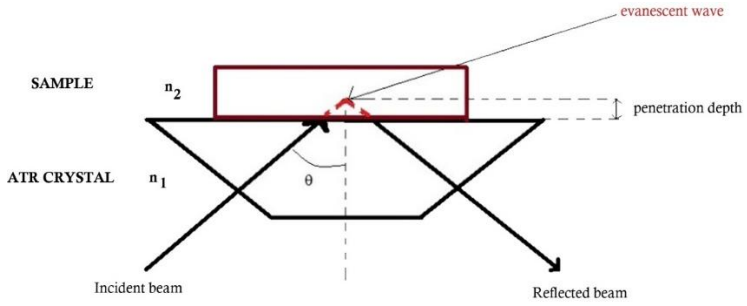

Fig. 2-9: Representation of a multiple reflection ATR-FTIR system [128]

Attenuated total reflectance fourier transform infrared spectroscopy (ATR-FTIR) measures total internal reflection created at the interface between two media based on refractive indices difference. One of the media is a ATR crystal (often used Germanium or Zinc/Selenide) and the other is the sample as it shown in Fig. 2.9. When an incident IR beam coming from ATR crystal reach to the less dense sample surface, material selectively absorbs the radiation at the surface area (up to 5 μm depth), therefore beam loses energy at those wavelengths and attenuated radiation plots as a function of wavelength by spectrometer [129].

Surface chemistry examination to obtain changes in chemical compositions of the samples Nicolet iS5 (Thermo Scientific, USA) single beam fourier transform infrared spectroscope (FTIR) equipped with iD5 attenuated total reflectance (ATR) was used. Collected spectra recorded between 400 and 4000 cm^{-1} wavelength with a resolution of 2 cm^{-1} for 64 scans using a ZnSe crystal which was placed to an incident angle of 45º.

2.4.5 Surface chemical analysis by X-ray photoelectron spectroscopy

X-ray photoelectron spectroscopy (XPS) is also known as Electron spectroscopy for chemical anaylysis (ESCA). It is widely used technique to investigate existing chemical elements with their quantity up to 10 nm depth of the sample surface by measuring the energy of emitted electrons from the surface. XPS also provides binding energy and density of the electronic states, chemical states, empirical formula, as well as determining thickness and its uniformity and detecting contaminations on the surface.

XPS basically consists of X-ray radiation source (generally doft X-rays of AlK_α with hυ = 1486.6 eV and MgK_α with hυ = 1253.6 eV) and a detector with an electron energy analyser (Fig. 2.10) which measure the kinetic energy of the emitted electrons from the surface by incident X-rays according the formula of;

$$E_k = h\upsilon - E_b - W \qquad (2)$$

where, E_k is kinetic energy of the emitted electrons, hυ is energy of the photons (from X-ray source), E_b is binding energy of electron and W is work function of the spectrometer. Therefore, analyzed data by electron spectrometer, presented by means of intensity versus electron energy in a graph. TFA XPS with a Multipak software (Physical Electronics) was used for XPS analysis.

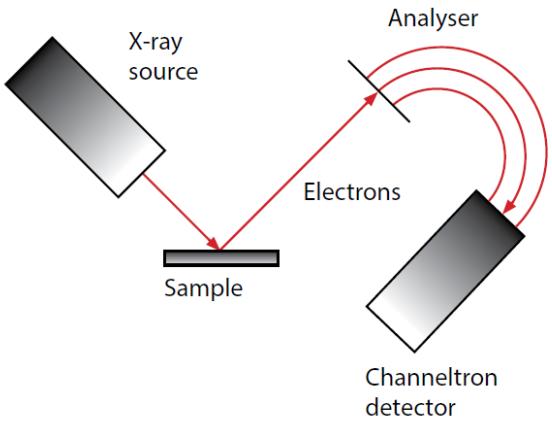

Fig. 2-10: XPS diagram.

The chemical composition of the surfaces was analyzed with X-ray photoelectron spectroscopy (XPS) using TFA XPS (Physical Electronics, USA). The samples exposed to X-rays over a 400 μm spot size with a monochromatic Al $K_{\alpha 1,2}$ radiation at 1486.6 eV, under 6×10^{-8} Pa chamber pressure. The emitted photoelectrons were detected with a hemispherical analyzer placed at angle of 45° in order to correlate to the normal plane of the samples. Survey-scan spectra were made with a 0.4 eV step resolution at 187.85 eV of pass energy. Surface neutralization was carried out by electron gun and MultiPak (Version 7.3.1) software (Physical Electronics, USA) was used to analyze elemental concentration.

2.4.6 Antibacterial activity test

Before antibacterial testing, samples were disinfected by rinsing with 70% denatured ethanol. For the determination two bacterial strains were used, gram-negative *Escherichia coli* (CCM 4517) and gram-positive *Staphylococcus aureus* (CCM 4517). The antibacterial testing was performed according to ISO 22196 with modifications. Bacterial suspensions (*E. coli* 1.4×10^7 CFU mL^{-1}; *S. aureus* 4.6×10^6 CFU mL^{-1}) were prepared in 1/500 Nutrient broth (Hi Media laboratories, India). The bacterial suspension was dispensed on the sample surface (dimensions 25×25 mm) in the volume 100 μL and the sample was covered with the polypropylene foil (dimensions 20×20 mm). Samples with foils were cultivated at 35 °C and 100 % relative humidity for 24 hours. After the incubation time, polypropylene foil was removed and each sample was completely washed by SCDLP broth (HiMedia laboratories, India), which was subsequently collected. The viable bacteria count was determined by the pour plate culture method (PCA, HiMedia laboratories, India).

2.4.7 Cell adhesion and proliferation test

Prior to in-vitro testing, the samples were disinfected by 70% ethanol. Cell reaction testing was performed using primary mouse embryonic fibroblast (Dr. Jiří Pacherník). The ATCC–formulated Dulbecco's Modified Eagle's Medium (BioSera, France) containing 20% of calf serum (BioSera, France),100 U mL^{-1} Penicillin/Streptomycin (GE Healthcare HyClone, United Kingdom) and 7μL L^{-1} mercaptoethanol (Serva, Germany), was used as the culture medium. Cells were seeded onto samples in concentration 1×10^5 cells per mL. As a reference, tissue polystyrene was used. To assess cell response, the fluorescence staining was carried out after 72 h of cell cultivation on the samples. All the tests were performed in quadruplicates. DNA staining with Hoechst 33258 (Invitrogen, USA) and actin filaments staining ActinRed™ 555 (Thermo Fisher Scientific, USA) was used to determine cell morphology. Before staining, cells were fixed and permeabilized. Cells were fixed using 4% formaldehyde (Penta, Czech

Republic) for 15 minutes, washed by PBS and subsequently poured with 0,5% Triton X-100 (Sigma-Aldrich, USA) for 5 minutes to permeabilization. After this time, cells were washed 3 times by PBS (Invitrogen, USA). Required amount of PBS, two drops per 1 mL of ActinRed™ 555 and 5µg mL^{-1} of Hoechst 33258 were added and left incubate for 30 minutes in the dark. Morphology of cells was observed and micrographs were taken using an inverted Olympus phase contrast microscope (IX 81, Japan).

2.4.8 Anticoagulation activity test

For anticoagulation tests, the blood was obtained by venous punction in accordance with the Helsinki Declaration and filled into blood collection tubes (5 mL each). Obtained human blood plasma was treated with 3.2 % citric acid (109 mmol/L) and then centrifuged at 3000 g for 15 min at room temperature. Anticoagulant activity determined by means of prothrombin time (PT), thrombin time (TT) and activated partial thromboplastin time (aPTT) using a SYSMEXCA - 1500 (Siemens, Germany) instrument. Each of the samples was examined three times.

3. RESULTS AND DISCUSSIONS

3.1 Antibacterial surface results

3.1.1 Surface wettability behaviour

Surface wettability of the sample surfaces was determined by static contact angle measurement with distilled water as a testing liquid and results are pointed out in Table 3.1. Water contact angle (Q_w) of PE was measured as 91.9° referred to a hydrophobic nature as its higher than 90°. It is one of the biggest drawback of PE for chemical agent immobilization which had to be applied surface treatment to change the hydrophobic nature to hydrophilic one. After MW plasma treatment, Q_w was drastically decreased to 55.8° which implies increased wettability and hydrophilicity by physico-chemical etching of the surface and also due to hydrophilic oxidative functional groups introduced by plasma[9]. Moreover, such decrease in Q_w refers increased wettability, therefore PE surface after plasma treatment become more convenient for further immobilization. Immobilized FQs onto PE_Tre sample at pH value of 3, namely SpF3, EnF3 and LmF3, slightly increased the Q_w to 63.7°, 61.3° and 59.9°, respectively. It is related to hydrophilic/hydrophobic natures of the immobilized FQs and also due to the coverage of cavities created by plasma etching by FQs. By means of FQs immobilization at pH value of 6, namely SpF6, EnF6 and LmF6, similar behavior obtained as in pH 3 counterparts: 65.3°, 62.9° and 63.4°, respectively. Nevertheless, Q_w values for pH 6 are slightly higher than that of Q_w values for pH 3 as a result of pH influence on FQs immobilization. Therefore, FQs immobilized samples at pH 6 value expected to show evenly higher antibacterial effect against selected bacterial strains, which is correlated by antibacterial tests, XPS results and SEM observations, discussed across following chapters.

Table 3.1. Water contact angle values.

PE	PE_Tre	SpF3	EnF3	LmF3	SpF6	EnF6	LmF6
91.9 ± 0.8	55.8 ± 1.1	63.7 ± 0.6	61.3 ± 2.3	59.9 ± 1.3	65.3 ± 2.6	62.9 ± 2.2	63.4 ± 2.1

3.1.2 Fourier transform infrared spectra investigations

Attenuated total reflectance Fourier transform infrared spectra (ATR-FTIR) of the samples have been investigated for mid-IR range of 4000 – 400 cm^{-1} revealed the typical major peaks for related wavenumbers for polyethylene by following: C-H stretching peaks at the wavenumbers of 2915 and 2850 cm^{-1} related to aliphatic hydrocarbon chain; C-H bending deformation peak at 1470 cm^{-1} and C-H methylene rocking deformation peak at the 719 cm^{-1} [27,122]. All studied samples showed typical major peaks of polyethylene, as a result of penetration depth of used ATR crystal ZnSe of 0.6 µm which was more thicker than FQs coated layers. Thus, collected data were more related to PE itself than to FQs layers. Therefore, only the wavenumber range of 1400 – 1000 cm^{-1} has been plotted in detail, which represents C-F stretching vibration (Fig.3.1) to reveal carbon-fluorine bond[13]. As it is seen in Fig. 3.1, PE before and after plasma treatment did not showed corresponding peak as expected, owing to nonexistence of fluorine content in neither PE nor plasma particles. In the case of SpF3 sample, corresponding peak was not observed, referring either lack of or insufficient immobilization. This is in agreement with XPS data, SEM morphology analysis and insufficient antibacterial activity. Rest of the FQs immobilized samples showed increased intensity as it pointed by arrows in Fig. 3, which correlates with other results and antibacterial activity assay. It is worth noting that peak intensity of EnF3 and EnF6 samples are adequate for most immobilized antibacterial agents, and also it should be mentioned that enrofloxacin only have a single fluorine substituent at C-6 position, while sparfloxacin and lomefloxacin has two fluorine substituents at their C-4 and C-6 positions, as it seen in Fig. 3.1.

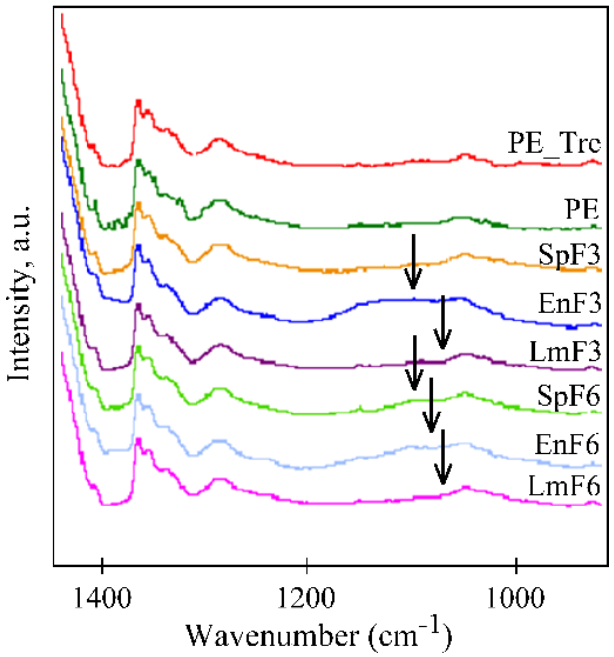

Fig. 3-1: ATR-FTIR results of selected area for C-F stretching.

3.1.3 X-ray photoelectron spectroscopy investigations

X-ray photoelectron spectroscopy (XPS) was used to reveal chemical surface compositions of samples and results are listed in Table 3.2. Carbon level is highest for the PE surface and decreased after plasma treatment as expected due to presence of oxide functional groups. Therefore, the oxygen level is increased regarding XPS spectra. Nitrogen content was not detected for the PE sample and amount of 4.2% was observed due to free amine groups created by air plasma treatment. Same phenomena was observed for carbon, oxygen and nitrogen levels of FQs immobilized samples since each of them has been exposed to plasma treatment before immobilization. Fluorine content of FQs immobilized samples is the critical point to prove successful bonding. As it is seen from the results, SpF3 sample did not show a fluorine content, which correlates with FTIR spectra, referring as insufficient boding. However, immobilization was successful for SpF6 sample as an evidence of fluorine level of 1.2 due to the higher pH value of 6 for immobilization step. EnF3 and LmF3 samples showed 0.9 and 0.7% of fluorine levels, respectively. In the case of EnF6 and LmF6 samples, observed fluorine levels were slightly higher than that of their pH 3 analogues as a results of applied pH for immobilization step. It should be noted that even if the monitored fluorine levels are close to each other, fluorine functionality for enrofloxacin is twice less than in case of sparfloxacin and lomefloxacin. Besides antibacterial activities of each FQs deposited samples, according to their fluorine levels, it is expected to perform higher antibacterial activity for enrofloxacin as described in following section.

Table 3.2. XPS results.

Samples	C1s%	O1s%	N1s%	F1s%	O1s/C1s	N1s/C1s	F1s/C1s
PE	99.3	0.7	-	-	0.007	-	-
PE_Tre	77.2	18.6	4.2	-	0.24	0.05	-
SpF3	77.1	18.6	4.3	-	0.24	0.05	-
EnF3	76.5	15.2	7.4	0.9	0.19	0.09	0.01
LmF3	76.2	16.2	6.9	0.7	0.21	0.09	0.009
SpF6	74.7	15.9	8.2	1.2	0.21	0.10	0.01
EnF6	76.7	15.0	7.3	1.0	0.19	0.09	0.01
LmF6	76.6	15.5	6.8	1.1	0.20	0.08	0.01

3.1.4 Scanning Electron Microscopy characterizations

Scanning electron microscope (SEM) was used to determine surface morphologies of the samples without conductive coating by using a low vacuum detector, obtained images are shown Fig.3.2. PE surface displayed relatively smooth surface with a minor wave-like morphology (Fig. 3.2a), as a result of PE foil processing. After plasma treatment, surface morphology became rougher due to the etching by plasma particles (Fig. 3.2b). According to the insufficient immobilization of sparloxacin, SpF3 sample monitored small fraction of sparfloxacin as marked in Fig.3.2c. However, for SpF6, sparfloxacin fraction is homogenously distributed to whole surface (Fig.4f). Enrofloxacin immobilization was successful and homogenously distributed to surface for both EnF3 and EnF6 samples, as it seen in Fig.3.2d and g. In the case of lomefloxacin immobilized samples, LmF6 surface screened more homogenously distributed fractions than that of LmF3 analogue, as depicted in Fig. 3.2h and e, respectively. SEM results well correlate with both FTIR and XPS chemical analysis.

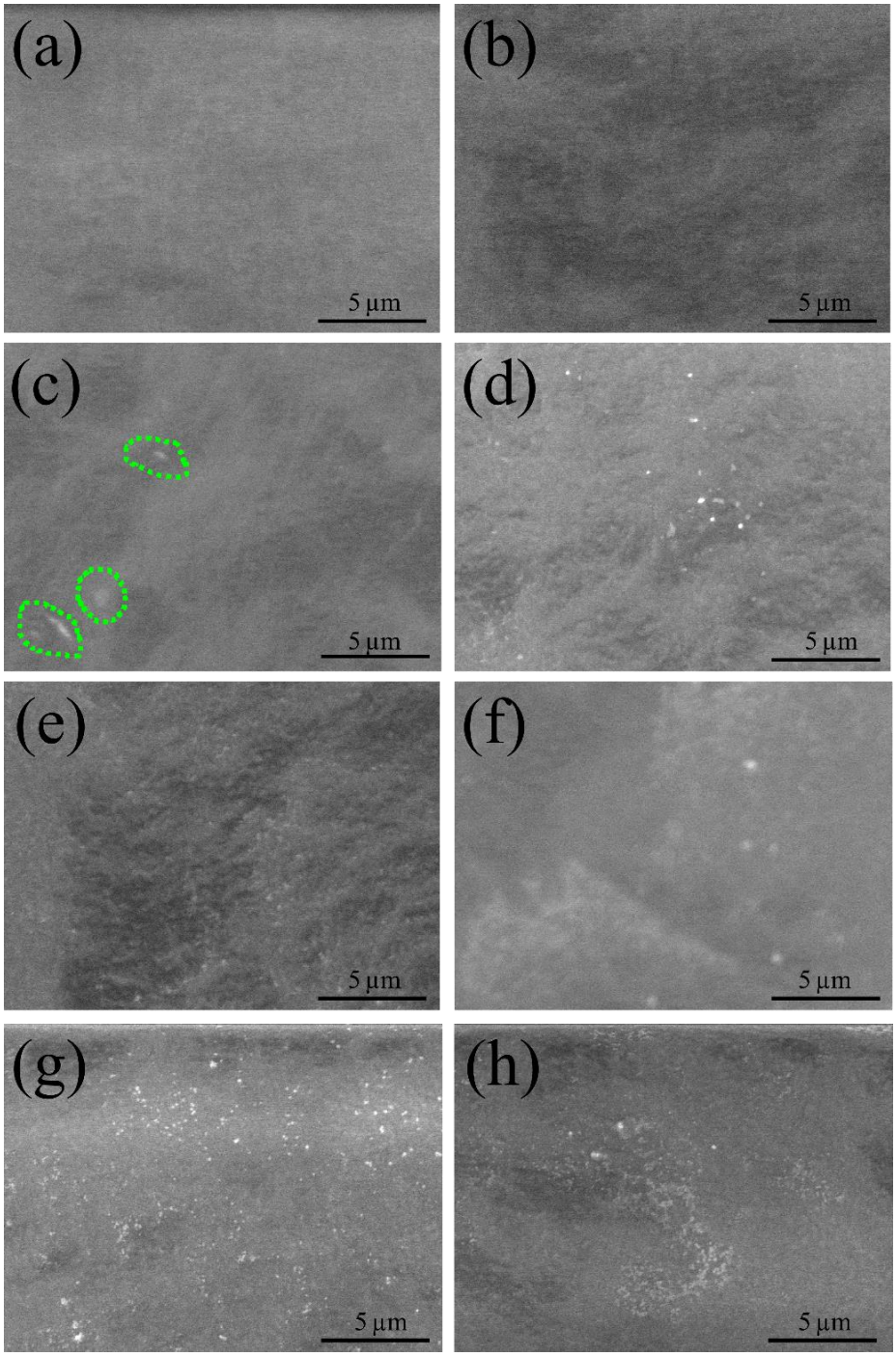

Fig. 3-2: SEM morphology results.

3.1.5 Antibacterial activity results

Antibacterial activity results against *S. aureus* (gram positive) and *E. coli* (gram negative) strains after 24 h of incubation time for all samples are listed in Table 3.3 with representative petri-dishes in Fig. 3.3. Reference PE sample did not show any antibacterial effect against both *S. aureus* and *E. coli* strains, as expected. Furthermore, none of the FQs immobilized samples displayed antibacterial activity against *S. aureus* strain but all were effective to *E. coli* strain except SpF3 sample, due to its insufficient immobilization at pH3, which was revealed by previous analysis methods, even so counted viable bacteria level is almost twenty times lower than PE sample. Beside, SpF6 sample was found effective, so then importance of pH on immobilization and antibacterial activity became evident.

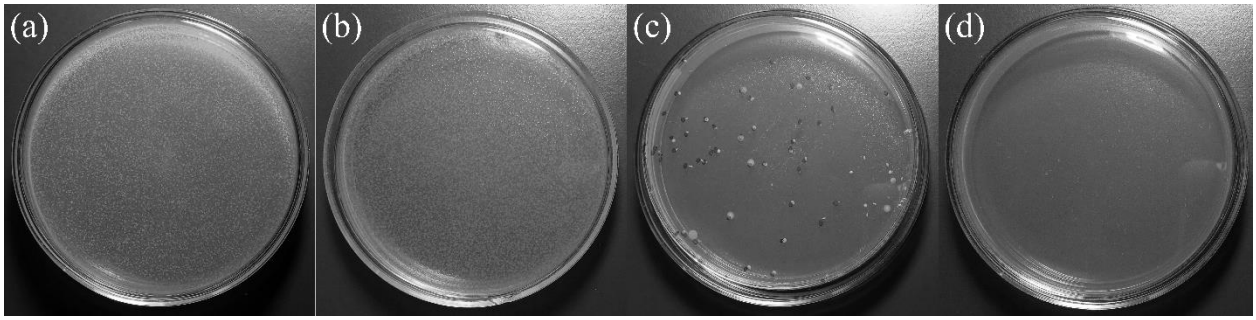

Fig. 3-3: Representative petri-dishes showing the lowering the amount of bacteria from left to right, after 24 hours incubation: a) S. aureus on EnF3, b) E. coli on SpF3, c) E. coli on LmF3 and d) E. coli on EnF3.

Both enrofloxacin immobilized samples of EnF3 and EnF6 were displayed the most effective antibacterial activity against *E. coli* strain, which was already expected by XPS results that they have the highest fluorine content. It indicates, not only the pH effect but chemical compositions of FQs also plays a paramount importance. In the case of lomefloxacin immobilized samples, LmF6 sample was displayed almost twenty times effective than LmF3 analogue. As a conclusion of pH comparison, it was revealed that samples of pH 6 were more effective against *E. coli* compare to their pH 3 analogues. Also, antibacterial activity against *S. aureus* and *E. coli* depends on their cell-wall compositions and physicochemical characteristics with an efficiency of each FQs against them. Therefore, level of successfully immobilized FQs onto the PE surface is the key point which is related to pH and concentration (w/v %) of the prepared chemical FQs solutions; surface characteristics of treated PE surface, such as wettability, roughness, charge density, functionality related to plasma treatment parameters (i.e., reactor type, generator power, frequency, used gas type and its flow rate, processing time, etc), and also environmental conditions, such as temperature and moisture.

Table 3.3. Antibacterial assay results by means of number of viable adhered bacteria.

Samples	***S. aureus*, N (CFU/cm^2)**	***E. coli*, N (CFU/cm^2)**
PE	3.0×10^5	4.3×10^6
PE_Tre	5.6×10^5	2.4×10^5
SpF3	3.2×10^5	< 1
EnF3	5.8×10^5	4.9×10^1
LmF3	2.0×10^5	4.4
SpF6	9.5×10^4	< 1
EnF6	3.6×10^5	2.5
LmF6	3.0×10^5	4.3×10^6

3.1.6 Conclusions

Antibacterial effect against both gram positive and negative strains of three different fluoroquinolones of sparfloxacin, enrofloxacin and lomefloxacin immobilization at different pH values onto low density polyethylene after air plasma treatment followed by N-allylmethylamine (MAAM) grafting has been studied. Antibacterial effect of enrofloxacin immobilized samples has been found out the best among other FQs immobilized samples independent of pH difference. SpF3 sample showed the worse effect but its effect was still twenty times higher than reference PE sample. SpF6 sample was much more effective, as it clearly seen in Table 3.3, owing to pH difference. Antibacterial effect of lomefloxacin immobilized sample at pH 6 has been found out more effective than its pH 3 analogue, such in sparfloxacin immobilized samples. Therefore, it should be noted that the antibacterial activity is higher at higher pH value due to more successful immobilizations of FQs, as also revealed by means of FTIR, XPS and SEM.

3.2 Cell interaction results

3.2.1 Surface wettability behaviour

The wettability of the surfaces, as a measure of its hydrophilicity, was determined by water contact angle (Q_w) measurement performed by sessile drop technique and results are listed in Table 3.4. Measure of the spread water on the surface refers its hydrophilicity and it depends on the interactions of the charges of the water molecules with the polar contents of the surface. Water contact angle recorded for PE surface is 85.3º, which is highly hydrophobic due to the typical characteristic of all polyolefins. After applied oxygen plasma treatment, hydrophilicity is significantly increased by decreasing Q_w to 57.3º for PERF sample due to the incorporation of oxidative functional groups [59] and tailoring the surface morphology by plasma particles. Therefore, PERF surface become more likely to interact further chemical modifications thanks to its higher surface energy. Furthermore, hydrophilic character of the surface is beneficial for cell adhesion/proliferation [21,130-132]. ChS immobilized samples onto grafted polymer brushes showed around 70º of water contact angle which is more hydrophobic compare to PERF due to wettability properties of ChS itself.

Table 3.4. Water contact angle and surface average roughness results.

Samples	**PE**	**PERF**	**PERFAC**	**PERFDC**	**PERFMC**
Contact Angle (θ°)	85.3 ±3.4	57.3 ± 14.4	70.3 ± 3.1	70.3 ± 14.9	69.3 ± 3.9
Surface roughness (nm)	11.1	14	13.4	11.2	13.9

3.2.2 Fourier transform infrared spectra investigations

Attenuated total reflectance fourier transform infrared spectra (ATR-FTIR) examination to characterize chemical changes in the near surface area of the samples was carried out for reference PE and ChS immobilized samples. As it clearly seen in Fig. 3.4, C-H stretching peaks are visible at the wavenumbers of 2915 and 2850 cm^{-1} for all samples, regarding to aliphatic hydrocarbon chain [54]. C-H bending deformation signal appears at the wavenumber of 1470 cm^{-1} and C-H methylene rocking deformation signal at the 719 cm^{-1} [53]. Amine groups which is introduced by oxygen plasma treatment seen at the wavenumber of 1630 cm^{-1} in addition to appeared common C-H spectra, except untreated PE sample. This indicates that not all of the amine groups were bonded and some of them are still exist even after ChS immobilization. Lastly, C-N stretch spectra is appeared at the wavenumber of 1100 cm^{-1} for ChS immobilized samples, as a results of aliphatic amines [74].

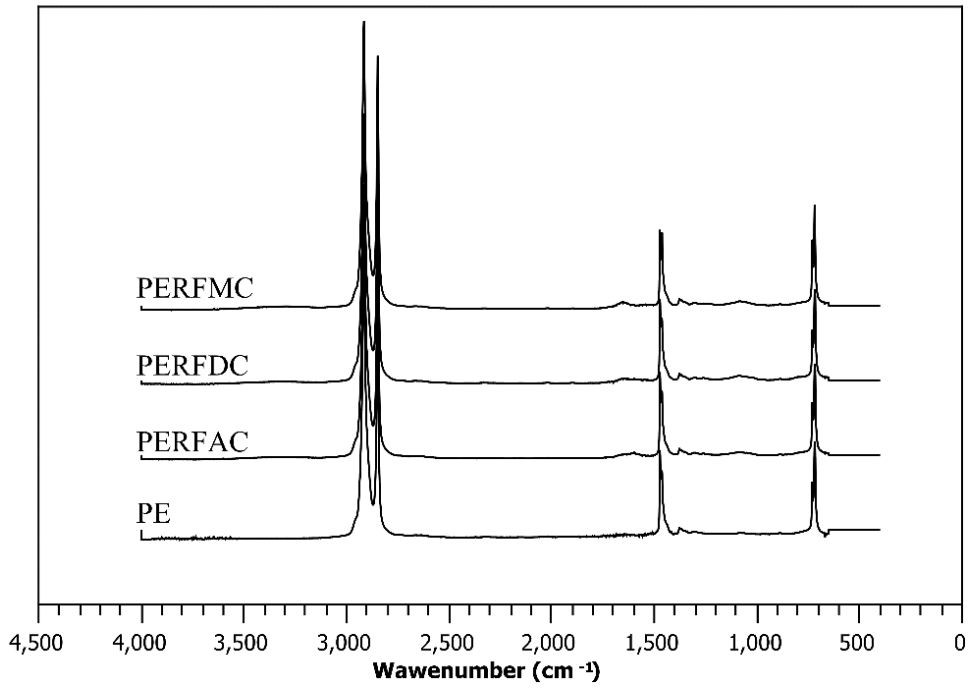

Fig. 3-4: ATR-FTIR spectra of reference and ChS immobilized samples.

3.2.3 X-ray photoelectron spectroscopy investigations

Chemical surface compositions of all studied samples were investigated by X-ray photoelectron spectroscopy (XPS) and results are listed in Table 3.5. Surface of reference PE sample have the maximum carbon level of 98.8% with a minimum oxygen level of 0.2%. Chemical composition of the sample after RF plasma treatment (PERF) revealed that oxygen level is significantly increased up to 31% thanks to introduced oxide groups by oxygen plasma and carbon level consequently decreased. Existence of 1.8% nitrogen level refers the free amine groups created by RF plasma treatment. Nevertheless, highest nitrogen contents were found for AAM, MAAM and DAAM grafted samples with the levels of 4.8, 4.6 and 4.4%, respectively. This is an evidence that three of used monomers were successfully grafted onto RF plasma treated samples and the maximum amine groups presence estimated by the highest nitrogen level of PERFA. In the case of ChS immobilized samples, nitrogen content decreased compare to monomer grafted samples as a result of bonding of amine groups with the ChS and increase in oxygen level due to presence of oxygen in the ChS. Appearing almost the same amount of sulphur contents also indicates successfully immobilized ChS layer onto AAM, MAAM and DAAM polymer brushes.

Table 3.5. Elemental compositions of the samples surfaces obtained by XPS.

Samples	C1s%	O1s%	N1s%	S2p%	O1s/C1s	N1s/C1s	S2p/C1s
PE	99.8	0.2	-	-	0.00	-	-
PERF	67.2	31	1.8	-	0.46	0.03	-
PERFA	72.6	22.6	4.8	-	0.31	0.07	-
PERFAC	62.3	31.3	2.8	3.6	0.50	0.04	0.06
PERFD	78.2	17.4	4.4	-	0.22	0.06	-
PERFDC	59.2	34	3	3.8	0.57	0.05	0.06
PERFM	75.3	20.1	4.6	-	0.27	0.06	-
PERFMC	60.1	33.3	2.9	3.7	0.55	0.05	0.06

3.2.4 Scanning electron microscopy characterizations

Surface morphologies were determined by means of scanning electron microscope performed with a low vacuum detector without conductive coating of the samples and showed in Fig. 3.5. Reference PE sample surface exhibit rather smooth and micron sized wave-like morphology. Among the ChS immobilized surfaces, PERFAC showed the most homogeneously distributed ChS layer which allows homogeneous adhesion of the fibroblast cells and continuously proliferation. In the case of PERFDC and PERFMC surfaces, ChS immobilization is not homogenous and rather less amount of ChS monitored on the PERFDC (Fig. 3.5c) and clustered ChS layers are clearly seen on Fig. 3.5d for the PERFMC surface. Such non-homogeneous immobilization may result in decrease of the cell adhesion and also it is a drawback for cell proliferation that it may occur limited on the ChS immobilized clusters and it also effects the cell morphology as discussed within this paper later on.

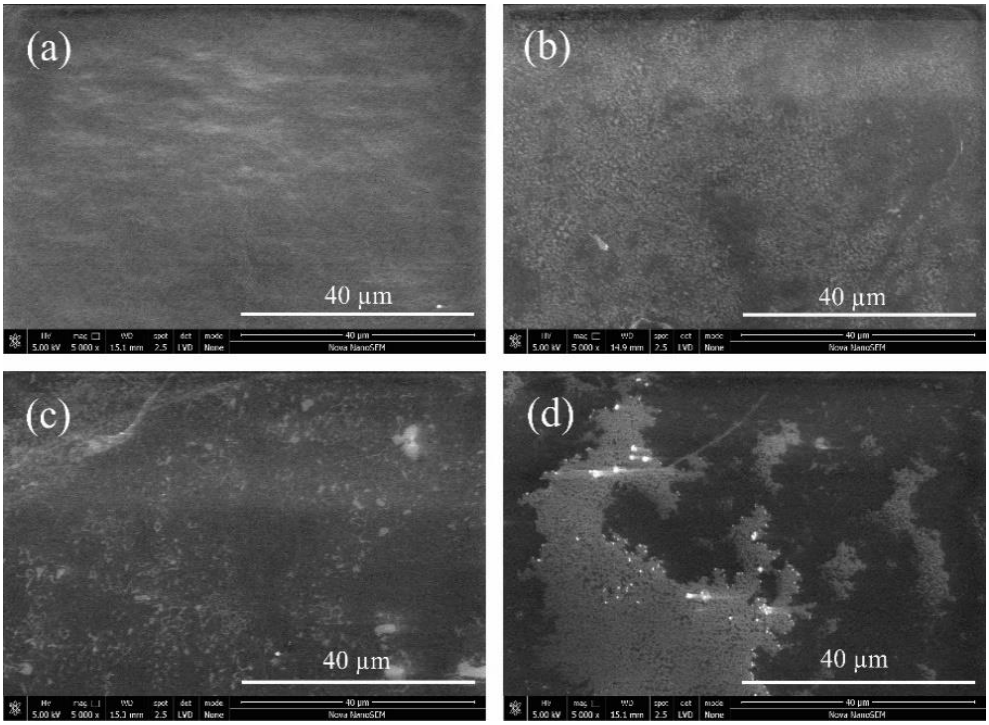

Fig. 3-5: Scanning electron microscopy results: a) PE, b) PERFAC, c) PERFDC, d) PERFMC.

3.2.5 Atomic force microscopy characterizations

Topographical changes of the modified samples investigated by atomic force microscope are shown in Fig. 3.6. Initially, PE surface is rather smooth with a grain-like structure and its average surface roughness is 11.1 nm (see Table 3.4). Plasma treatment was resulted increased average surface roughness to 17.6 nm via etching the surface by plasma particles. ChS immobilized samples onto AAM, MAAM and DAAM polymer brushes were decreased the average surface roughness by lowered the porosity, introduced by surface etching, to 13.4, 13.9 and 14.3 nm, respectively. There is no significant difference compare to each other, however, they are still rougher than PE sample in contrast to SEM micrographs discussed in section 3.2.4. Thus, increased surface area was obtained for further cell adhesion.

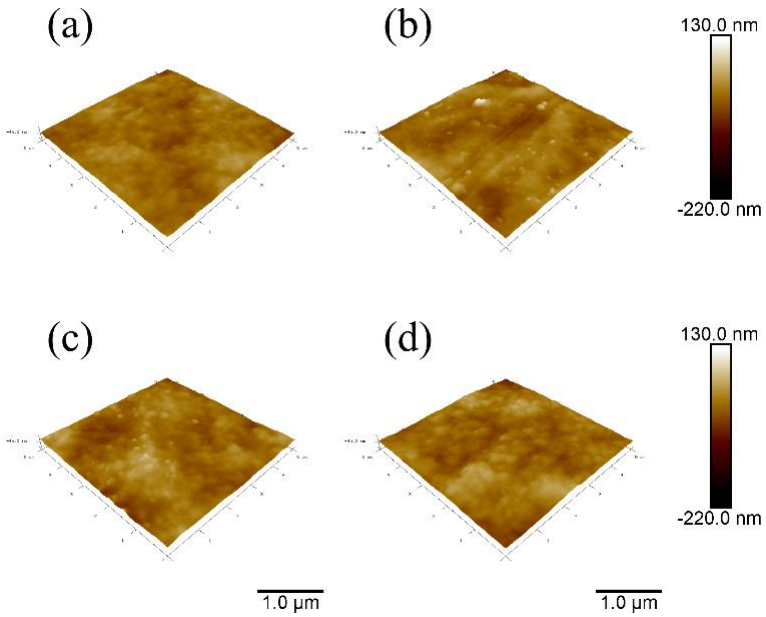

Fig. 3-6: Atomic force microscopy results: a) PE, b) PERFAC, c) PERFDC, d) PERFMC.

3.2.6 Cell morphology and proliferation investigations

Biological response to any material is influenced by various material characterizations. Hydrophobicity, roughness, homogeneity or functional groups belong to important surface properties affecting cell adhesion and proliferation. Therefore, surface modifications are consider as desirable for many materials used in biomedicine or tissue engineering, which are not suitable for this utilization in native form. This was also confirmed in this study, as can be seen on Fig. 3.7. On the reference PE (Fig. 3.8b), cells were not growing homogenously as they made clusters and moreover the actin filaments were missing. Hence, the reference PE was assigned as non cytocompatible sample. However, as was presumed, after surface modification of PE cell compatibility was significantly increased. The cell quantity was comparable to the reference on samples PERFAC, PERFMC and PERFDC (Fig. 3.8c and e). Nevertheless, there are visible changes in cell morphology on the samples PERFMC and PERFDC. As was mentioned, cell behavior on the surface is affected by several surface properties. As the chemical composition, hydrophilicity and porosity of samples with immobilized ChS were almost the same, they did not caused changes in cell morphology between these samples. The morphological changes are probably effected by the surface homogeneity, which was confirmed by SEM investigations. The most homogenous ChS layer was detected on the sample PERFAC, where the most physiological cell behavior was observed. Thus, the sample PERFAC was evaluated as the best cytocompatible sample.

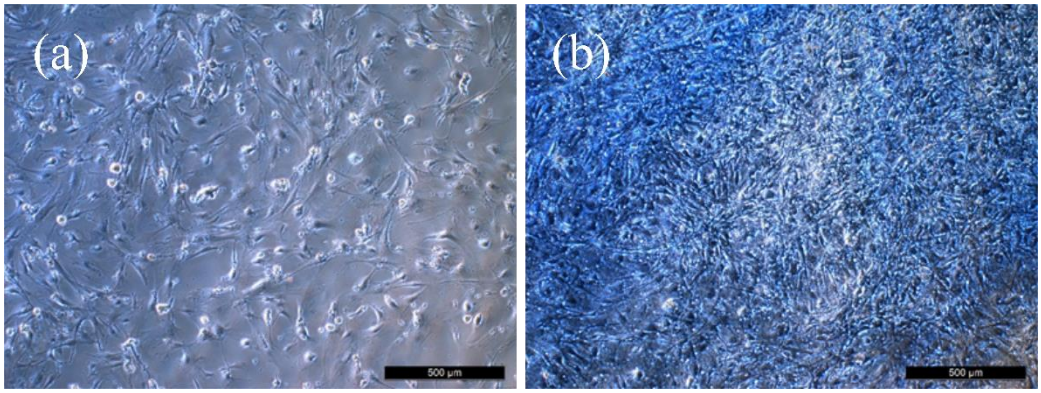

Fig. 3-7: Mouse primary fibroblast cells after 48 hours, a) LDPE, b) bioactive LDPE

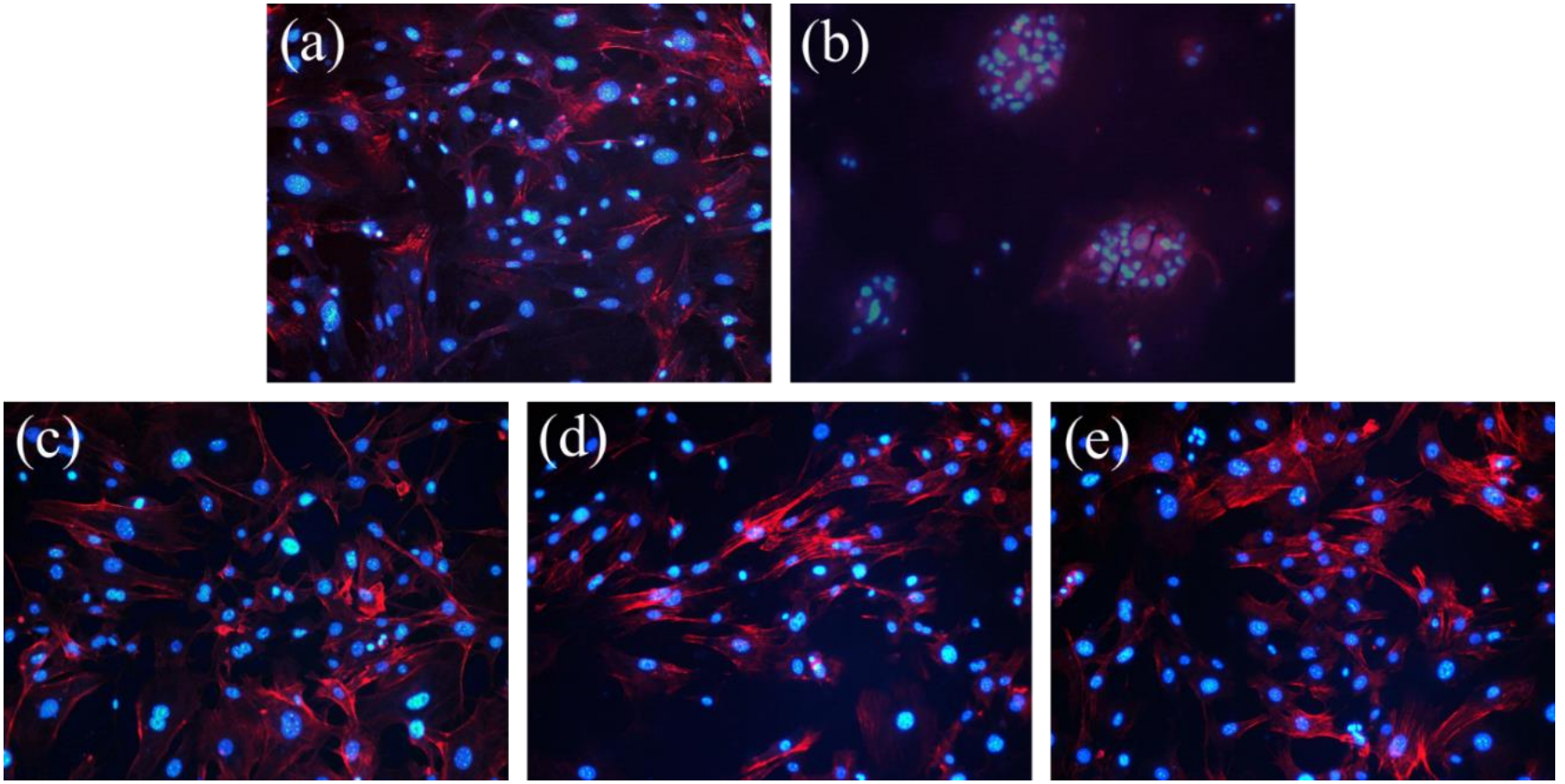

Fig. 3-8: Fibroblast proliferation on examined samples: a) Reference Polystyrene (PS), b) Reference Polyethylene (PE), c) PERFAC, d) PERFDC, e) PERFMC.

3.2.7 Conclusions

A multistep method for chondroitin sulfate immobilization onto plasma treatment applied and polymer brushes grafted low density polyethylene has been subjected to fibroblast cells to compare cell proliferations. Plasma treatment was enhanced the surface wettability via physical etching and oxidative functional groups and provided functionalized surface for further AAM, MAAM and DAAM polymer brushes grafting. ChS successfully immobilized onto those polymer brushes as it provided by XPS but more homogeneous coating was occurred for PERFAC sample as it seen from SEM results. Cell proliferation did not monitored on the reference PE sample as expected. All of the ChS immobilized samples were showed that cell compatibility was significantly enhanced as a result of existence of ChS. Besides, cell morphology for PERFAC sample was found the most cytocompatible due to the homogeneously distributed ChS layer. Nevertheless, it is still comparable to PERFMC and PERFDC samples.

3.3 Blood thrombosis results

Due to the similar and unsatisfying anticoagulation behaviours of AAM, MAAM and DAAM immobilized samples, their investigations except anticoagulation test is not placed in this paper. In fact, as it seen after surface examinations, existing of their polymer brushes has been revealed, however, did not show significant effect onto further heparin/fucoidan immobilization.

3.3.1 Surface wettability behaviour

Surface wettability was carried out by static contact angle measurement by sessile drop technique using distilled water as a testing liquid and results are given in Table 3.6. Changes in the water contact angle (θ_w) by spreading the water on the surface, as a result of bonding interactions of the water molecules, refers hydrophilicity, which has a correlation with its surface energy. Water contact angle of the reference PE is drastically decreased of about 33% after plasma treatment from 85.3° to 56.9° as a result of plasma induced hydrophilic oxidative functional groups presence [59] and also with respect to the surface roughening which is discussed in section 3.3.5. Thus, wettability and surface energy is increased and therefore PET surface become more likely to interact with further modifications. It has been also known that such hydrophilic surfaces have significant influence on the cell and blood plasma protein interactions.

Heparin and fucoidan immobilized samples before plasma treatment (PEH and PEF, respectively) and heparin immobilized sample after plasma treatment (PETH) showed almost the same wettability properties (Table 3.6) due to insufficient immobilization of anticoagulants, which is discussed in section 3.3.4. Fucoidan immobilized sample after plasma treatment (PETF) showed the lowest water contact angle value among all anticoagulant immobilized samples which corresponds to its hydrophilic character. It indicates that fucoidan was successfully immobilized onto the surface as also demonstrated by scanning electron microscopy.

Table 3.6. Water contact angle and average surface roughness values.

Samples	**PE**	**PEH**	**PEF**	**PET**	**PETH**
Contact Angle (θ°)	85.3 (±3.4)	81.9 (±12.2)	79.4 (±8.3)	56.9 (±11)	79.6 (±5.3)
Surface roughness (nm)	24.2	13.9	20.3	46.7	17.9

3.3.2 Fourier transform infrared spectra investigations

Fig. 3.9. Shows chemical changes in the near-surface area of the selected samples obtained by attenuated total reflectance fourier transform infrared (ATR-FTIR) spectra. Spectrum of the PE, main peaks at the wavenumbers of 2915 cm^{-1} and 2850 cm^{-1} are ascribed to C-H stretching are visible (typical for aliphatic hydrocarbon chain) [54]. Characteristic signals of C-H bending deformation and methylene rocking deformation are appeared at the wavenumbers of 1470 cm^{-1} and 719 cm^{-1}, respectively. By means of the PET sample, in addition to PE peaks, amine groups introduced by plasma treatment is clearly seen at the wavenumber of 1630 cm^{-1} and oxygen containing hydroperoxides at 3370 cm^{-1} [53]. PEH and PEF spectra did not show significant changes on the peaks than PET peaks, which means peaks at the 3370 cm^{-1} and 1630 cm^{-1} are still visible, as a evidence of the limited immobilization (or no immobilization at all, but some seen on SEM images) of the heparin and fucoidan onto PE surface without plasma treatment. In the case of PETH and PETF samples, hydroperoxide and amine group peaks are not visible anymore as an indicator of heparin and fucoidan immobilization onto PE surface after plasma treatment.

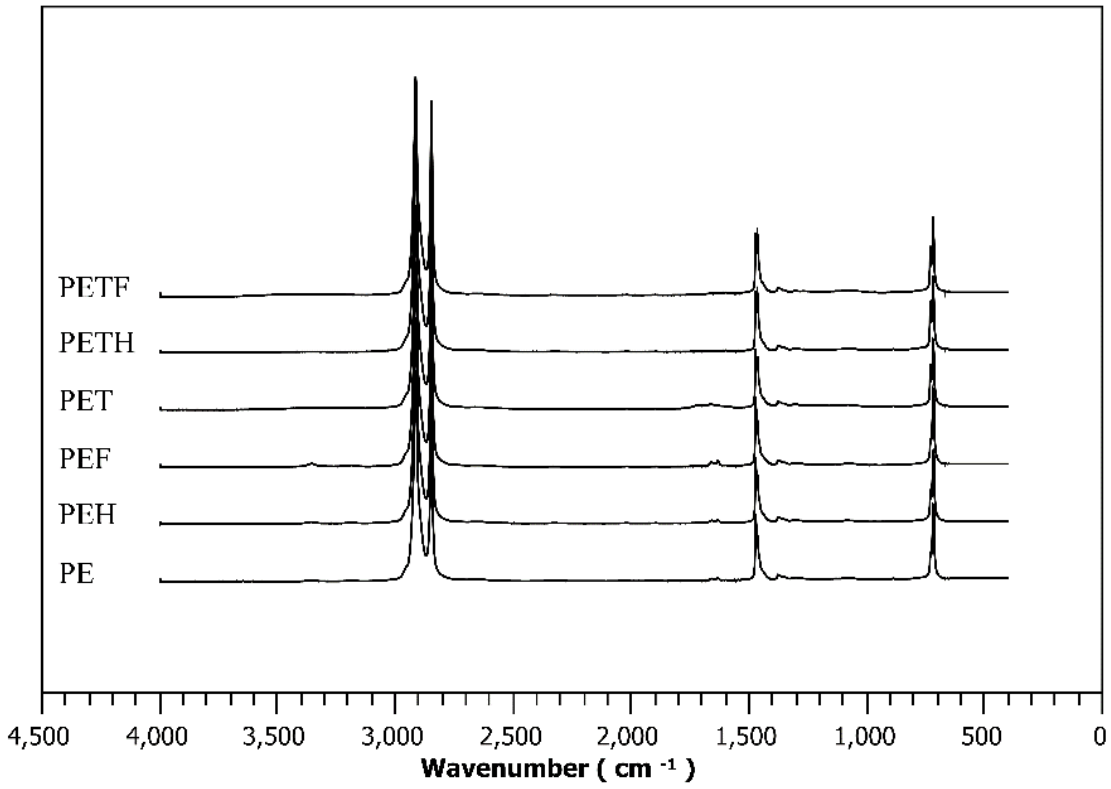

Fig. 3-9: ATR-FTIR spectra.

3.3.3 X-ray photoelectron spectroscopy investigations

Chemical surface compositions are listed in Table 3.7. By means of samples without plasma treatment (PE, PEH, PEF) were displayed the same carbon, oxygen, nitrogen and sulphur level regarding XPS spectra, irrelevant to exposure of anticoagulants. Constant carbon levels referred to as immobilization of heparin/fucoidan did not take place on the PE surface, or it was limited since no apparent carbon consumption by introducing of anticoagulants as we have witnessed partially heparin/fucoidan layers on the surfaces by SEM in Fig. 3.10. As expected, oxygen level is also constant which means none of the oxide groups by plasma treatment were introduced yet and absence of nitrogen is evidence of free amine groups.

After plasma treatment, carbon level is decreased about 12 % and significantly increased oxygen content due to the introduced oxides groups; likewise appearing nitrogen level of 1.1 % means introduction of amine groups by plasma treatment.

Anticoagulant immobilization after plasma treatment, in the case of PETH and PETF, decrease the carbon and nitrogen level (amine groups) by chemical bonding and increase the oxygen content due to the oxide containing functional groups presence in heparin and fucoidan. Besides consumption of carbon and nitrogen, existing pH levels are the most important evidence of anticoagulants immobilization. According to the pH levels, PETF has 2.4 % of pH which is almost 12 times higher than pH content of PETH by 0.2 %. Immobilized fucoidan layer onto the PET sample is greater than that of immobilized heparin layer, by considering the pH levels. Therefore, it has more significant impact on antithrombotic properties.

Table 3.7. Elemental compositions and ratios of the tested surfaces obtained by XPS.

Samples	C1s%	O1s%	N1s%	S2p%	O1s/C1s	N1s/C1s	S2p/C1s
PE	98.7	1.3	-	-	0.013	-	-
PEH	98.6	1.4	-	-	0.014	-	-
PEF	98.7	1.3	-	-	0.013	-	-
PET	87.2	11.7	1.1	-	0.134	0.013	-
PETH	85.7	13.3	0.8	0.2	0.155	0.009	0.0023
PETF	82.3	14.8	0.5	2.4	0.18	0.006	0.0292

3.3.4 Scanning electron microscopy characterizations

Surface morphological images of the samples taken by SEM are shown in Fig. 3.10. Reference PE sample exhibit homogenous, relatively smooth surface morphology with a minor uniform fiber-like feature stem from the production (Fig. 3.10a). After plasma treatment, surface morphology became rougher due to the surface reorganization by exposition in plasma (Fig. 3.10b). As a result, increased surface area was generated by increasing roughness, which is a desired surface condition for further interactions. Anticoagulants of heparin and fucoidan immobilization to PE without plasma treatment is limited (Fig. 3.10c and d) and partially attached on the PE surface, which means allows direct contact of the blood with the substrate. Thus, limited antithrombotic effect is expected. Plasma modified samples (Fig. 3.10e and f) exhibit more homogenous and completely coated layers thanks to the functional groups introduced by plasma treatment. Beside antithrombotic activities of anticoagulants, bonding type is also crucial for further interactions with the blood discussed in section 3.3.6.

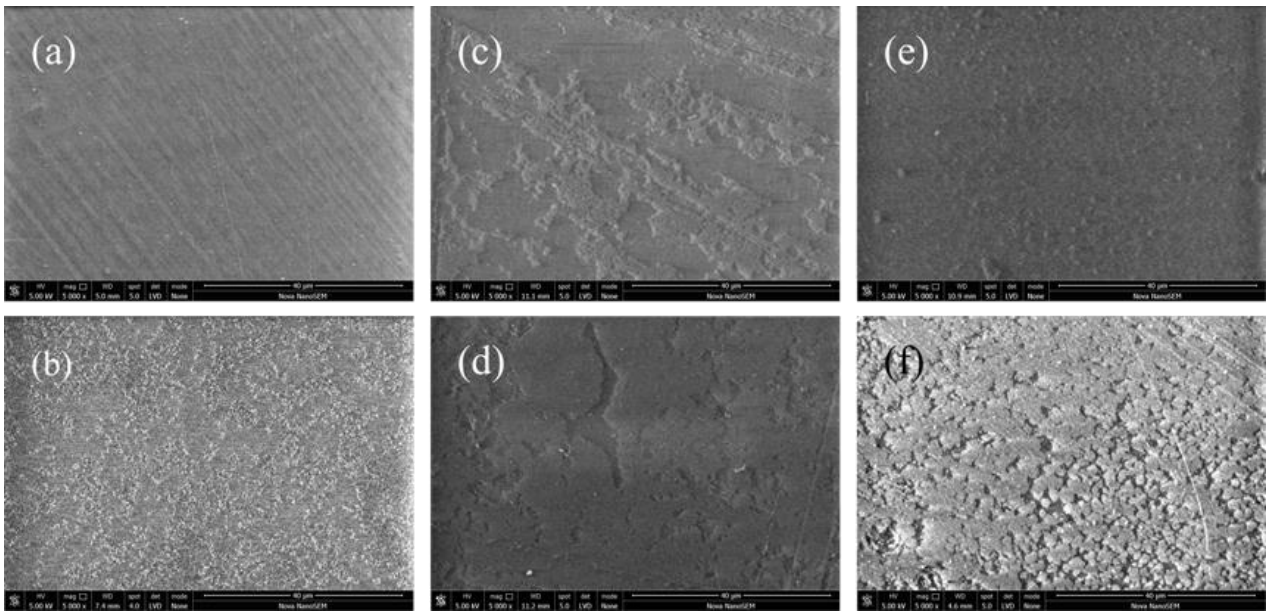

Fig. 3-10: Surface morphology by SEM: (a) PE; (b) PET; c) PEH; (d) PEF; (e) PETH and (f) PETF.

3.3.5 Atomic force microscopy characterizations

Surface topography investigations of the samples were carried out by AFM are shown in Fig. 3.11. Surface roughness of the reference PE sample is rather smooth with a average roughness value of 24.2 nm (Table 3.6). Direct immobilizations of anticoagulants heparin and fucoidan onto the PE surface decreased the surface roughness to 13.9 and 20.3 nm, respectively (Fig. 3.11c and d). This indicates heparin and fucoidan particles slightly covered the pores of PE with a non-uniform feature. However, PE surface became more rough (46.7 nm) after plasma treatment by etching of the surface (Fig. 3.11b). Similar behaviour was observed for PETH and PETF samples after heparin and fucoidan immobilization that layers of anticoagulants decreased the roughness of PET to 17.9 and 24 nm, respectively. Besides, the roughness of the PETH and PETF is slightly higher than their PEH and PEF counterparts which indicate the effect of plasma treatment. It should be emphasized that roughness value itself is not an indicator of uniformity of the coated layer but also features of the immobilized anticoagulants should be take into consideration. Moreover, although reference PE and PETF shows similar surface roughness values, their wettability behaviours are completely different as it discussed previously in section 3.3.1. Therefore, it is evident that wettability, even in the same roughness conditions, is also related to chemical features of the examined substrates. Roughness is an important feature by means of contact area for cell and blood protein adhesion.

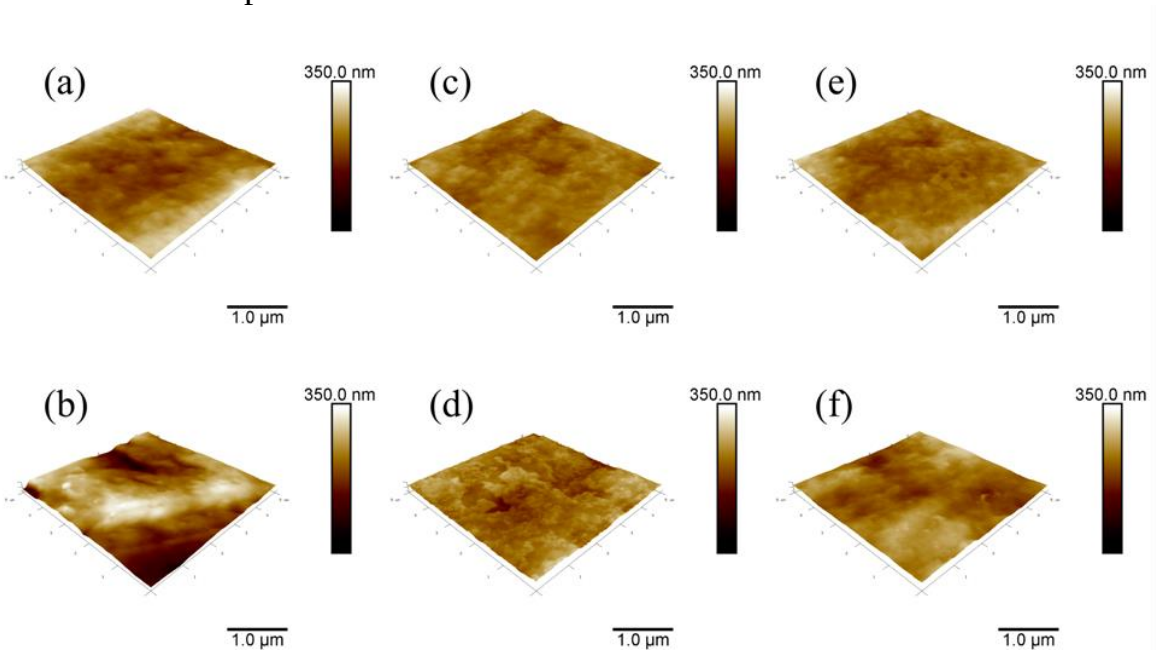

Fig. 3-11: Surface topography by AFM : (a) PE; (b) PET; (c) PEH; (d) PEF; (e) PETH and (f) PETF.

3.3.6 Anticoagulation activity studied in Vitro

The blood coagulation cascade consist of tissue-mediated extrinsic pathway, surface-mediated intrinsic pathway and common coagulation pathway [133]. Examination of those coagulation pathways for all samples were carried out by means of prothrombin time (PT), activated partial thromboplastin time (aPTT) and thrombin time (TT).

PT assay measures the clot formation time in extrinsic and common coagulation pathway. Normal time range for healthy donors is generally altering between11 – 13.5 sec [54]. As summarized in Table 3.8, all samples are within this range.

aPTT assay is related to intrinsic and common coagulation pathway. Between 25 and 32 seconds is the range of aPTT for healthy donors [134]. As it summarized in Table 3, none of the samples performed anticoagulant activity on intrinsic pathway since none of them prolonged aPTT which indicates no remarkable inhibition of intrinsic pathway factors. Although, some of the samples exhibited slightly lower aPTT values than the control value, it can be ignored since the difference is still under the physiological threshold.

TT is a measure of thrombin formation time by transferring fibrinogen to fibrin in the common coagulation pathway [133]. The lower limit for TT to perform anticoagulation activity is 20 sec [134]. TT of the control PE value (15.9 sec) is comparable to TT value of the plasma modified sample (PET), that indicates plasma induced oxidative functional groups and its increased hydrophilicity were not influenced TT in contrast with the fact that hydrophilicity affects the coagulation cascade, but it is worth noting that pH values and the surface charges are another two crucial parameters to affect coagulation cascade. Similar results were observed for AAM, MAAM, DAAM grafted samples (PETA, PETM and PETD, respectively) with their anticoagulant immobilized counterparts: Antithrombotic activity was not monitored (Table 3.8) since intramolecular interactions between grafted polymer brushes and anticoagulants were not sufficient for immobilization what is in agreement with SEM investigations.

Table 3.8. Antithrombotic test results. PT: Prothrombin time; aPTT: activated partial thromboplastin time; TT: Thrombin time.

Samples	PT (sec)	aPTT (sec)	TT (sec)
PE (Control)	11.5	24.8	15.9
PEH	11.5	25	16.5
PEF	11.2	24.1	16.8
PET	11.4	25.9	16.8
PETH	11.5	25.7	16.6
PETF	10.9	27.3	20.9
PETA	11.5	25.2	16.3
PETAH	11.6	26.5	17.2
PETAF	11.1	26.5	18
PETM	11.7	26.4	16.9
PETMH	11.4	25.2	16.7
PETMF	11	24.2	16.5
PETD	11.4	24.2	16.6
PETDH	11.4	24.1	16.5
PETDF	11	27.1	19.5

Immobilizations of anticoagulants heparin and fucoidan without plasma treatment (PEH and PEF) did not affect TT due to the fact that insufficient immobilization as it seen in Fig. 4. This is consistent with XPS results that PEH and PEF samples did not posses sulphur content to increase heparin cofactor II (HCII) based antithrombotic activity [109,122]. Due to the fact that sulphur content observed only for heparin and fucoidan immobilized samples after plasma treatment (PETH and PETF), expected antithrombotic activity observed only in the case of PETF with a TT value of 20.9 sec. First and foremost is its sulphur content is 2.4 %, while PETH's is only 0.2 %.

Secondly, fucoidan immobilization onto PET surface was sufficiently homogeneous than heparin immobilized PETH sample as it seen in Fig. 3.10, therefore its anticoagulation activity is increased, besides, heparin is covalently bonded and did not interact sufficiently with the coagulation factors.

3.3.7 Conclusions

Plasma treatment onto LDPE and its effect of further anticoagulant heparin and fucoidan immobilizations with anticoagulation behaviours has been studied. Surface wettability of the surface remarkably increased by introduced oxidative functional groups by plasma treatment and increased surface roughness by etching simultaneously as it revealed by contact angle measurement and AFM. Anticoagulant immobilization onto LDPE surface without plasma treatment was not sufficient as it seen on SEM images and XPS results but treated surfaces showed more homogeneous layers and sulphur content, especially fucoidan immobilization was more successful than heparin. Anticoagulation tests revealed that PETF sample has anticoagulation effect since its more homogeneous immobilization and also higher anticoagulation effect of fucoidan than heparin. Hence, surface modification LDPE by plasma treatment following by fucoidan immobilization found out an effective route to prevent surface mediated thrombus formations of blood contacting biomaterial.

SUMMARY

Bioactive polymer surfaces has been created onto one of the most used polymer of low density polyethylene (LDPE) to overcome three drawbacks of:

- Biomaterial induced infection
- Lack of cellular interaction of biomaterials with the implanted living tissue
- Biomaterial induced blood thrombus.

Due to the fact that highly hydrophobic nature and unsufficient surface energy of LDPE, plasma treatment have been applied onto their surface to functionalize the surface by means of physical etching and introducing oxidative functional groups by plasma particles. Three different plasma reactors of DC, RF and MW were used for surface functionalization and it has been relieved that all plasma types were sufficiently functionalized the LDPE surface in a minute, without major differencies which have been relieved out by wettability test, atomic force microscope investigations and X-ray photoelectron spectroscopy (XPS).

Increasing chemical bonding of used specific bioactive agents onto the polymeric substrate, polymer brushes of three amine group containing monomers of allylamine (AAM), N,N-dimethylallylamine (DAAM) and N-allylmethylamine (MAAM) have been created onto the functionalized surface by plasma process by means of co-polymerization process. However, no significant difference have been detected regarding to further immobilization, which indicates, each of the primary, secondary and tertiary amine group containing monomers were sufficiently worked to make covalent immobilization with the bioactive chemical agents.

Bioactive LDPE surfaces to overcome mentioned three drawback of polymeric biomaterials, were successfully accomplished for each purpose by multi-step approach, as it revealed by XPS and SEM investigations.

However, effect of FQs has been founded limited with the gram negative *E. coli* strain and did not exhibit significant effect on gram positive *S. aureus* strain. Owing to the fact that, each of the FQs agents is effective to wide range of both gram negative and gram positive strains (as it revealed by means of their oral usage), reason of the ineffectiveness might be the inadequate density of the FQs solutions, which may need to be increased the weight/volume ratio.

In the case of ChS containing bioactive surface, cellular interactions were sufficient, however, effect of AAM, DAAM and MAAM was not clearly seen, therefore their grafting might be improved by increasing the exposure time to obtain higher concentration or creating more homogeneously polymer brushes. Furthermore, as it revealed by SEM, immobilization of ChS was not homogenous, therefore it effects cellular response and cell morphology.

Lastly, difference of heparin and fucoidan immobilized bioactive surfaces was clearly seen by performed tests. Even if effect of fucoidan was higher than that of heparin to blood coagulation, it was slightly across the limit of anti-thrombus effect. The reason might be the ineligible pH of the solutions which have an important effect on immobilization and blood protein interactions. Altering the pH might be the good way to reveal out the differences of anti-thrombotic performance.

CONTRIBUTIONS TO SCIENCE AND PRACTICE

The presented thesis brings new aspects to bioactive polymer systems used in biomedical applications. Biggest three drawbacks of polymeric biomaterials implanted into the body, has been studied to adequately improve biocompatibility, as follows:

Regulating surface topography, energy and chemistry of low density polyethylene by plasma treatment using different three kind of reactors was successfully accomplished to improve its further chemical immobilizations, moreover grafting polymers brushes of amine group containing monomers, as a mediator between LDPE surface and bioactive agents to improve chemical bonding.

Bioactive agents of fluoroquinolones, chondoitin sulfate, heparin and fucoidan were carefully selected according to their unique effects on bacterial, cellular and blood interaction and their efficiency has been proved.

Therefore, bioactive polymer systems for avoiding biomaterial induced bacterial infection and blood thrombus formation along with improved cellular interaction has been successfully obtained and contributed to science and practice.

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LIST OF ABBREVIATIONS

AAM	Allylamine
AC	Alternative current
AFM	Atomic force microscope
Al	Aluminium
APGD	Atmospheric pressure glow discharge
aPTT	Activated partial thromboplastin time
ASTM	American society for testing and materials
AT III	Antithrombin
ATR	Attenuated total reflectance
BA	bioactive agents
CASING	Crosslinking by activated species of inert gases
CCD	Charge coupled device
CFU	Colony forming unit
ChS	Chondoitin sulfate
ChS A	Chondroitin 4-sulfate
ChS B	Dermatan sulfate
ChS C	Chondroitin 6-Sulfate
Co	Cobalt
Cr	Chrome
DAAM	N,N-dimethylallylamine
DBD	Dielectric barrier discharge
DC	Direct current
DNA	Deoxyribonucleic acid
EnF	Enrofloxacin
ePTFE	Expanded Polytetrafluoroethylene
FU	Fucoidan
FTIR	Fourier transform infrared spectroscopy
FQs	Fluoroquinolones
g	Gram
GAGs	Glycosaminoglycans
GHz	Gigahertz
GPPS	General purpose of polystyrene
h	Hour
HCII	Heparin cofactor II
HDPE	High density polyethylene
HEP	Heparin
HIPS	High impact polystyrene
IR	Infrared
K	Kelvin
KHz	Kilohertz
L	Liter

LDPE	Low density polyethylene
LLDPE	Linear-low density polyethylene
LmF	Lomefloxacin
LMW	Low molecular weight
LVD	Low vacuum detector
m	Meter
MAAM	N-allylmethylamine
MDPE	Medium density polyethylene
Mg	Magnesium
MHZ	Megahertz
Min	Minute
mL	milliliter
mmol	Milimole
MRSA	Methicillin resistant *Staphylococcus aureus*
MW	Microwave
n_e	Electrons density
n_i	Ion density
nm	Nanometer
Pa	Pascal
PA	Polyamide
PC	Poly(carbonate)
PDMS	Polydimethylsiloxane
PE	Polyethylene
PEEK	Polyether ether ketone
PES	Polyether sulfone
PET	Poly(ethylene terephthalate)
PMMA	Polymethyl methacrylate
PP	Poly(propylene)
PS	Polystyrene
PT	Prothrombin time
PTFE	Poly(tetrafluoroethylene)
PU	Polyurethane
PVC	Polyvinyl chloride
θ_w	Water contact angle
R_a	Average surface roughness
RF	Radio frequency
RNA	Ribonucleic acid
S	Second
sccm	Standard cubic centimeter
Se	Selenide
SEM	Scanning electron microscope
SEE System	Surface energy evaluation system
SpF	Sparfloxacin
SPS	Syndiotactic polystyrene

T_e	Electron temperature
T_g	Gas temperature
T_i	Ion temperature
TT	Thrombin time
ULDPE	Ultra-low density polyethylene
UV	Ultraviolet
VLDPE	Very-low density polyethylene
W	Work function
w/v	Weight/Volume ratio
XPS	X-ray photoelectron spectroscopy
Zn	Zinc
µL	Microliter
µm	Micrometer
°C	Centigrade degree

REFERENCES

[1] C. A. C. Zavaglia and M. H. Prado da Silva, "Feature Article: Biomaterials," *Ref. Modul. Mater. Sci. Mater. Eng.*, vol. 10, pp. 1–5, 2016.

[2] A. Tathe, M. Ghodke, and A. Nikalje, "A brief review: biomaterials and their application," *Int. J. Pharm. Pharm. Sci.*, vol. 2, no. 4, pp. 19–23, 2010.

[3] Ebnesajjad, Sina and Kavyon Modjarrad. *Handbook of Polymer Applications in Medicine and Medical Devices*. 1st ed. Elsevier Inc.,2013. ISBN: 978-0-323-22805-3.

[4] Ehrenstein, W.Gottfried. *Polymeric materials.* Carl Hanser Verlag GmbH & Co. KG,2001. eISBN: 978-3-446-43413-4.

[5] M.F. Maitz, Applications of synthetic polymers in clinical medicine, *Biosurface and Biotribology*, vol. 1, 161-176, 2015.

[6] Young, R.J. and P.A. Lovell, *Polymers*, Chapman & Hall, 2nd ed. 1991. ISBN 0412 30640 9(PB).

[7] Bronzino JD, The biomedical engineering handbook, CRC Press LLC, 2nd edition, 2000, ISBN 084930461X.

[8] Callister WD, Materials science and engineering: An introduction, John Wiley & Sons, Inc. 7th edition. ISBN 9780471736967.

[9] Ebnesajjad, Sina and Kavyon Modjarrad. *Handbook of Polymer Applications in Medicine and Medical Devices*. 1st ed. Elsevier Inc.,2013. Chapter 3. Plastics used in medical devices, ISBN: 978-0-323-22805-3.

[10] P. F. F. Amaral, M. A. Z. Coelho, and J. A. P. Coutinho, "on Plasma Deposited Organosilicon Thin Films," vol. 56, pp. 1256–1262, 2006.

[11] M. Urbankova, M. Hrabalikova, I. Poljansek, N. Miskolczi, and V. Sedlarik, "Antibacterial polymer composites based on low-density polyethylene and essential oils immobilized on various solid carriers," *J. Appl. Polym. Sci.*, vol. 132, no. 47, pp. 1–9, 2015.

[12] Akay M, Introduction to polymer science and technology, BOOKBONN, 2012, ISBN 9788740300871

[13] Ebnesajjad, Sina and Kavyon Modjarrad. *Handbook of Polymer Applications in Medicine and Medical Devices*. 1st ed. Elsevier Inc.,2013. Chapter 10. Polymeric biomaterials, ISBN: 978-0-323-22805-3.

[14] Bronzino JD, The biomedical engineering handbook, CRC Press LLC, 2nd edition, 2000, Chapter 40, polymeric biomaterials, ISBN 084930461X.

[15] W. He and R. Benson, "10 - Polymeric Biomaterials," *Plast. Des. Libr.*, pp. 159–175, 2011.

[16] Ratner BD, Hoffman AS, Schoen FJ, Lemons JE, Biomaterials Science : An introduction to materials in medicine, Academic Press, USA, 1996, ISBN 0125824602

[17] SEDLARIK, Vladimir. *Biodegradation - life of science*. Rolando CHAMY a Francisca ROSENKRANZ. InTech, 2013 Antimicrobial Modification of Polymers. 187-378 s. ISBN 978-953-51-1154-2.

[18] WONG, J. Y., BRONZINO, J.D. *Biomaterials*. 1st ed. Boca Raton: CRC Press, 2007. ISBN 978-0-84-93-7888-1

[19] B. a. Jucker, H. Harms, and A. J. B. Zehnder, "Adhesion of the positively charged bacterium Stenotrophomonas (Xanthomonas) maltophilia 70401 to glass and teflon," *J. Bacteriol.*, vol. 178, no. 18, pp. 5472–5479, 1996.

[20] B. Nilsson, K. N. Ekdahl, T. E. Mollnes, and J. D. Lambris, "The role of complement in biomaterial-induced inflammation," *Mol. Immunol.*, vol. 44, no. 1–3, pp. 82–94, 2007.

[21] L. C. Xu and C. A. Siedlecki, "Effects of surface wettability and contact time on protein adhesion to biomaterial surfaces," *Biomaterials*, vol. 28, no. 22, pp. 3273–3283, 2007.

[22] L. C. Xu, J. W. Bauer, and C. A. Siedlecki, "Proteins, platelets, and blood coagulation at biomaterial interfaces," *Colloids Surfaces B Biointerfaces*, vol. 124, pp. 49–68, 2014.

[23] H. Chen, L. Yuan, W. Song, Z. Wu, and D. Li, "Biocompatible polymer materials: Role of protein-surface interactions," *Prog. Polym. Sci.*, vol. 33, no. 11, pp. 1059–1087, 2008.

[24] N. J. Kaleekkal, A. Thanigaivelan, M. Durga, R. Girish, D. Rana, P. Soundararajan, and D. Mohan, "Graphene Oxide Nanocomposite Incorporated Poly(ether imide) Mixed Matrix Membranes for in Vitro Evaluation of Its Efficacy in Blood Purification Applications," *Ind. Eng. Chem. Res.*, vol. 54, no. 32, pp. 7899–7913, 2015.

[25] I. Junkar, U. Cvelbar, and M. Lehocky, "Plasma Treatment of Biomedical Materials," vol. 45, no. 3, pp. 221–226, 2011.

[26] T. Desmet, T. Desmet, R. Morent, R. Morent, N. De Geyter, N. De Geyter, C. Leys, C. Leys, E. Schacht, E. Schacht, P. Dubruel, and P. Dubruel, "Nonthermal Plasma Technology as a Versatile Strategy for Polymeric Biomaterials Surface Modi cation: A Review," *Am. Chem. Soc.*, vol. 10, no. 9, 2009.

[27] D. Patel, J. Wu, P. Chan, S. Upreti, G. Turcotte, and T. Ye, "Surface modification of low density polyethylene films by homogeneous catalytic ozonation," *Chem. Eng. Res. Des.*, vol. 90, no. 11, pp. 1800–1806, 2012.

[28] M. Lehocký, L. Lapčík,Jr., M. C. Neves, T. Trindade, L. Szyk-Warszynska, P. Warszynski, and D. Hui, "Deposition/Detachment of Particles on Plasma Treated Polymer Surfaces," *Mater. Sci. Forum*, vol. 426–432, pp. 2533–2538, 2003.

[29] P. F. F. Amaral, J. M. da Silva, M. Lehocky, A. M. V. Barros-Timmons, M. A. Z. Coelho, I. M. Marrucho, and J. A. P. Coutinho, "Production and characterization of a bioemulsifier from Yarrowia lipolytica," *Process Biochem.*, vol. 41, no. 8, pp. 1894–1898, 2006.

[30] SODHI, R.N.S. Application of surface analytical and modification techniques to biomaterial research. *J. Electron Spectrosc. Relat. Phenom.* 1996, vol. 81,no. 3, p. 269-284.

[31] CHOI, D.M., PARK, C.K., CHO, K., PARK, C.E. Adhesion improvement of epoxy resin/polyethylene joints by plasma treatment of polyethylene. *Polymer* 1997, vol. 38, no. 25, p. 6243-6249.

[32] DE GEYTER, N., MORENT, R., LEYS, C. Penetration of a dielectric barrier discharge plasma into textile structures at medium pressure. *Plasma Sources Sci. Technol.* 2006, vol. 15, no. 1, p. 78-84.

[33] Roth, J.R., 1995. Industrial Plasma Engineering, Volume 1: Principles. Institute of Physics Publishing, ISBN 0-7503-0317-4, 538p. UK.

[34] Chen, F.F., 1984. Introduction to Plasma Physics and Controlled Fusion, Volume 1:Plasma Physics. Plenum Press, ISBN 0-306-41332-9, 421p. USA.

[35] Lieberman, M.A., Lichtenberg, A.J., 2005. Principles of Plasma Discharges and Material Processing. John Wiley & Sons, ISBN 0-471-72001-1,757p. USA.

[36] BISMARCK, A., BROSTOW, W., CHIU, R., LOBLAND, H.E.H., HO, K.K.C. Effects of plasma surface treatment on tribology of thermoplastic polymers. Polym. Eng. Sci. 2008, vol. 48, no. 10, p. 1971-1976.

[37] Borcia C, Borcia G, Dumitrascu N, Surface treatment of polymers by plasma and UV radiation, *Rom Journ Phys*, 2011, Vol. 56, No 1-2, 224-232.

[38] France RM, Short RD, Plasma treatment of polymers, *J Chem Soc, Faraday Trans*, 1997, 93(17), 3173-3178.

[39] J. M. Goddard and J. H. Hotchkiss, "Polymer surface modification for the attachment of bioactive compounds," *Prog. Polym. Sci.*, vol. 32, no. 7, pp. 698–725, 2007.

[40] Gao J, A novel technique for wastewater treatment by contact glow-discharge electrolysis, *Pakistan journal of biological sciences*, 2006, 9(2), 323-329.

[41] Friedrich J, The plasma chemisty of polymer surfaces, WILEY-VCH Verlag GmbH & Co. KGaA, 2012, Germany, ISBN 9783527318537.

[42] MRÁČEK, A., LEHOCKÝ, M., SMOLKA, P., GRULICH, O., VELEBNÝ, V. The allylamine grafting on the plasma pre-treated polyester nonwoven fabric: Preparation, characterization and utilization. *Fibers Polym.* 2011, vol.11, no. 8, p. 1106-1110.

[43] ALMAZÁN-ALMAZÁN, M.C., PAREDES, J.I., PÉREZ-MENDOZA, M., DOMINGO-GARCÍA, M., LÓPEZ-GARZÓN, F.J., MARTÍNEZ-ALONSO, A., TASCÓN, J.M.D. Surface characterisation of plasma-modified poly(ethylene terephthalate). *J. Colloid Interface Sci.* 2006, vol. 293, no. 2, p. 353–363.

[44] CVELBAR, U., MOZETIČ, M., KLANJŠEK-GUNDE, M. Selective oxygen plasma etching of coatings, *IEEE Trans. Plasma Sci.* 2005, vol. 33, no.2, p.236-237.

[45] SOWE, M., NOVÁK, I., VESEL, A., JUNKAR, I., LEHOCKÝ, M., SÁHA, P., CHODÁK, I. Analysis and characterization of printed plasma-treated polyvinyl chloride. *Int. J. Polym. Anal. Charact*. 2009, vol. 14, no. 7, p. 641-651.

[46] OLDE-RIEKERINK, M.B., TERLINGEN, J.G.A., ENGBERS, G.H.M., FEIJEN, F. Selective etching of semicrystalline polymers: CF4 gas plasma treatment of poly(ethylene). *Langmuir* 1999, vol. 15, no. 14, p. 4847-4856.

[47] Wolf R, Sparavigna AC, Role of surface treatment on wetting and adhesion, *Engineering*, 2010, 2, 397-402.

[48] Slepicka P, Kasalkova NS, Stranska E, Bacakova L, Svorcik V, Surface characterization of plasma treatment polymers for applications as biocompatible carriers, *eXPRESS Polymer Lettes*, 2013, Vol. 7, No 6, 535-545.

[49] Vidaurre EFC, Achete CA, Gallo F, Garcia D, Simao R, Habert AC, Surface modification of polymeric materials by plasma treatment, *Materials Research*, 2002, Vol. 5, No 1, 37-41.

[50] Hall JR, Westerdahl CAL, Devine AT, Bodnar MJ, Activated gas plasma surface treatment of polymers for adhesive bonding, *Journal of applied polymer science*, 1969, Vol. 13, 2085-2096.

[51] Schonhorn H, Hansen RH, Surface treatment of polymers for adhesive bonding, *Journal of applied polymer science*, 1967, Vol. 11, 1461-1474.

[52] A. Asadinezhad, I. Novák, M. Lehocký, V. Sedlařík, A. Vesel, I. Junkar, P. Sáha, and I. Chodák, "An in vitro bacterial adhesion assessment of surface-modified medical-grade PVC," *Colloids Surfaces B Biointerfaces*, vol. 77, no. 2, pp. 246–256, 2010.

[53] A. Popelka, I. Novák, M. Lehocký, I. Chodák, J. Sedliacik, M. Gajtanska, M. Sedliaciková, A. Vesel, I. Junkar, A. Kleinová, M. Špírková, and F. Bílek, "Anti-bacterial treatment of polyethylene by cold plasma for medical purposes," *Molecules*, vol. 17, no. 1, pp. 762–785, 2012.

[54] Wang J, Huang N, Yang P, et al. The effects of amorphous carbon films deposited on polyethylene terephthalate on bacterial adhesion. *Biomaterials*.2004;25(16):3163-3170.

[55] Zhang W, Chu PK, Ji J, et al. Plasma surface modification of poly vinyl chloride for improvement of antibacterial properties. *Biomaterials*. 2006;27(1):44-51. doi:10.1016/j.biomaterials.2005.05.067.

[56] Zhao L, Chu PK, Zhang Y, Wu Z. Antibacterial coatings on titanium implants. *J Biomed Mater Res - Part B Appl Biomater*. 2009;91(1):470-480. doi:10.1002/jbm.b.31463.

[57] Popovici D, Barzic AI, Hulubei C, et al. Surface properties and antibacterial testing of a partially alicyclic polyimide film modified by RF plasma and NaOH/AgNO3 treatment. *Polym Test*. 2016;49:94-99. doi:10.1016/j.polymertesting.2015.11.016.

[58] Speranza G, Gottardi G, Pederzolli C, et al. Role of chemical interactions in bacterial adhesion to polymer surfaces. *Biomaterials*. 2004;25(11):2029-2037. doi:10.1016/j.biomaterials.2003.08.061.

[59] Bílek F, Sulovská K, Lehocký M, et al. Preparation of active antibacterial LDPE surface through multistep physicochemical approach II: graft type effect on antibacterial properties. *Colloids Surf B Biointerfaces*. 2013;102:842-848. doi:10.1016/j.colsurfb.2012.08.026.

[60] Bílek F, Křížová T, Lehocký M. Preparation of active antibacterial LDPE surface through multistep physicochemical approach: I. Allylamine grafting, attachment of antibacterial agent and antibacterial activity assessment. *Colloids Surf B Biointerfaces*. 2011;88(1):440-447. doi:10.1016/j.colsurfb.2011.07.027.

[61] S. Ferraris and S. Spriano, "Antibacterial titanium surfaces for medical implants," *Mater. Sci. Eng. C*, vol. 61, pp. 965–978, 2016.

[62] K. Hori and S. Matsumoto, "Bacterial adhesion: From mechanism to control," *Biochem. Eng. J.*, vol. 48, no. 3, pp. 424–434, 2010.

[63] A. Asadinezhad, I. Novák, M. Lehocký, F. Bílek, A. Vesel, I. Junkar, P. Sáha, and A. Popelka, "Polysaccharides Coatings on Medical-Grade PVC: A Probe into Surface Characteristics and the Extent of Bacterial Adhesion," *Molecules*, vol. 15, no. 2, pp. 1007–1027, 2010.

[64] Kanematsu H. and Barry M.B., *Biofilm and materials science*, Springer, 2015, ISBN 978331914568.

[65] Sohn EH, Kim J, Kim BG, et al. Inhibition of bacterial adhesion on well ordered comb-like polymer surfaces. *Colloids Surfaces B Biointerfaces*. 2010;77(2):191-199. doi:10.1016/j.colsurfb.2010.01.023.

[66] CAO H., LIU, X. Silver Nanoparticles - modified Films versus Biomedical Device-Associated Infections, *WIREs Nanomedicine and Nanobiotechnology* 2010, vol. 2, p. 670.

[67] ZIEBUHR, W., HENNING, S., ECKART, M., KRÄNZLER, H., BATZILLA, C., KOZITSKAYA, S. Nosocomial infections by Staphylococcus Epidermidis: How a Commensal Bacterium Turns into a Pathogen,*International Journal of Antimicrobial Agents* 2006, vol. 28, p. 14.

[68] LEVISON, M. E. Pharmacodynamics of antimicrobial drugs. *Infectious Disease Clinics of North America*. 2004, vol. 18, no. 3, s. 451-65, vii. ISSN 0891-5520; 0891-5520.

[69] OUATTARA, B., SIMARD, R. E., PIETTE, G., BEGIN, A., HOLLEY, R. A. Inhibition of Surface Spoilage Bacteria in Processed Meats by Application of Antimicrobial Films Prepared with Chitosan, *Int J Food Microbiol* 2000, vol. 62, p. 139.

[70] OUATTARA, B., SIMARD, R. E., PIETTE, G., BEGIN, A., HOLLEY, R. A. Diffusion of Acetic and Propionic Acids from Chitosan – based Antimicrobial Packaging Films, *J Food Sci* 2000, vol. 65, p. 768.

[71] HIDALGO, E. a C. DOMINGUEZ. Mechanisms underlying chlorhexidine-induced cytotoxicity. *Toxicology in Vitro*. 2001, vol. 15, no. 4–5, s. 271-276. ISSN 0887-2333.

[72] DYNES, J. J. et al. Quantitative mapping of chlorhexidine in natural river biofilms. *The Science of the Total Environment*. 2006, vol. 369, no. 1-3, s. 369-383. ISSN 0048-9697; 0048-9697.

[73] A. Beberok, D. Wrzesniok, M. Otreba, and E. Buszman, "Impact of sparfloxacin on melanogenesis and antioxidant defense system in normal human melanocytes HEMa-LP - An in vitro study," *Pharmacol. Reports*, vol. 67, no. 1, pp. 38–43, 2015.

[74] Karbassi E, Asadinezhad A, Lehocký M, Humpolíček P, Sáha P. Bacteriostatic activity of fluoroquinolone coatings on polyethylene films. *Polym Bull*. 2015;72(8):2049-2058. doi:10.1007/s00289-015-1388-2.

[75] Zeng HJ, Yang R, Liu B, Lei LF, Li JJ, Qu LB. Simple and sensitive determination of sparfloxacin in pharmaceuticals and biological samples by immunoassay. *J Pharm Anal*. 2012;2(3):214-219. doi:10.1016/j.jpha.2012.02.001.

[76] Efthimiadou EK, Karaliota A, Psomas G. Mononuclear dioxomolybdenum(VI) complexes with the quinolones enrofloxacin and sparfloxacin: Synthesis, structure, antibacterial activity and interaction with DNA. *Polyhedron*. 2008;27(1):349-356. doi:10.1016/j.poly.2007.09.013.

[77] Komarnicka UK, Starosta R, Guz-Regner K, Bugla-Płoskońska G, Kyzioł A, Jeżowska-Bojczuk M. Phosphine derivatives of sparfloxacin – Synthesis, structures and in vitro activity. *J Mol Struct*. 2015;1096:55-63. doi:10.1016/j.molstruc.2015.04.044.

[78] C. Piras, A. Soggiu, V. Greco, P. Anna, F. Del, L. Putignani, A. Urbani, J. E. Nally, L. Bonizzi, and P. Roncada, "Mechanisms of antibiotic resistance to enro fl oxacin in uropathogenic Escherichia coli in dog ☆," *J. Proteomics*, vol. 127, pp. 365–376, 2015.

[79] H. Gupta, M. Aqil, R. K. Khar, A. Ali, A. Bhatnagar, and G. Mittal, "Sparfloxacin-loaded PLGA nanoparticles for sustained ocular drug delivery," *Nanomedicine Nanotechnology, Biol. Med.*, vol. 6, no. 2, pp. 324–333, 2010.

[80] Attili AR, Preziuso S, Ngu Ngwa V, Cantalamessa A, Moriconi M, Cuteri V. Clinical evaluation of the use of enrofloxacin against Staphylococcus aureus clinical mastitis in sheep. *Small Rumin Res*. 2016;136:72-77.

[81] Karademir U, Boyacioglu M, Kum C, Sekkin S. Comparative pharmacokinetics of enrofloxacin , danofloxacin and marbofloxacin following intramuscular administration in sheep. 2015;133:108-111.

[82] Kumar GP, Phani AR, Prasad RGS V, et al. Polyvinylpyrrolidone oral fi lms of enro fl oxacin : Film characterization and drug release. *Elsevier BV*. 2014;471(1-2):146-152. doi:10.1016/j.ijpharm.2014.05.033.

[83] Gbylik-Sikorska M, Posyniak A, Sniegocki T, et al. Influence of enrofloxacin traces in drinking water to doxycycline tissue pharmacokinetics in healthy and infected by Mycoplasma gallisepticum broiler chickens. *Food Chem Toxicol*. 2016;90:123-129. doi:10.1016/j.fct.2016.02.006.

[84] P. Fernandes, I. Sousa, L. Cunha-Silva, M. Ferreira, B. De Castro, E. F. Pereira, M. J. Feio, and P. Gameiro, "Synthesis, characterization and antibacterial studies of a copper(II) lomefloxacin ternary complex," *J. Inorg. Biochem.*, vol. 131, pp. 21–29, 2014.

[85] Chen Z, Wang Z, Fu Q, Fang P, He C. Microstructure and Surface State of Plasma-treated High-density Polyethylene Elucidated by Energy-tunable Positron Annihilation and Water Contact Angle Measurements. 2014;2:1-5.

[86] Lehocký M, Lapčík,Jr. L, Neves MC, et al. Deposition/Detachment of Particles on Plasma Treated Polymer Surfaces. *Mater Sci Forum*. 2003;426-432:2533-2538. doi:10.4028/www.scientific.net/MSF.426-432.2533.

[87] Lopez-Garcia J, Bilek F, Lehocky M, Junkar I, Mozetic M, Sowe M. Enhanced printability of polyethylene through air plasma treatment. *Vacuum*. 2013;95:43-49. doi:10.1016/j.vacuum.2013.02.008.

[88] X. Liu and P. X. Ma, Polymeric scaffolds for bone tissue engineering, *Ann. Biomed. Eng.*, vol. 32, no. 3, pp. 477–486, 2004.

[89] M. Jafari, Z. Paknejad, M. R. Rad, S. R. Motamedian, M. J. Eghbal, N. Nadjmi, and A. Khojasteh, "Polymeric scaffolds in tissue engineering: A literature review," *J. Biomed. Mater. Res. - Part B Appl. Biomater.*, pp. 2015–2016, 2015.

[90] Ke C, Chena J, Guo Y, Chen ZW, Cai J, Migration mechanism of mesenchymal stem cells studied by QD/NSOM, *Biochimica et Biophysica Acta*, 2015, 1848, 859-868.

[91] V. V. K. Vendra, L. Wu, and S. Krishnan, *Polymer Thin Films for Biomedical Applications*, vol. 5. 2007.

[92] M. Tengdelius, C.-J. Lee, M. Grenegård, M. Griffith, P. Påhlsson, and P. Konradsson, "Synthesis and biological evaluation of fucoidan-mimetic glycopolymers through cyanoxyl-mediated free-radical polymerization.," *Biomacromolecules*, vol. 15, no. 7, pp. 2359–68, 2014.

[93] L.-Y. Huang and M.-C. Yang, “Surface immobilization of chondroitin 6-sulfate/heparin multilayer on stainless steel for developing drug-eluting coronary stents,” *Colloids Surfaces B Biointerfaces*, vol. 61, no. 1, pp. 43–52, 2008.

[94] E. György, A. Pérez del Pino, J. Roqueta, C. Sánchez, and A. G. Oliva, “Processing and immobilization of chondroitin-4-sulphate by UV laser radiation,” *Colloids Surfaces B Biointerfaces*, vol. 104, pp. 169–173, 2013.

[95] Y. Ni, Z. Tang, W. Cao, H. Lin, Y. Fan, L. Guo, and X. Zhang, “Tough and elastic hydrogel of hyaluronic acid and chondroitin sulfate as potential cell scaffold materials,” *Int. J. Biol. Macromol.*, vol. 74, pp. 367–375, 2015.

[96] W. Schneiders, C. Rentsch, S. Rehberg, S. Rein, H. Zwipp, and S. Rammelt, “Effect of chondroitin sulfate on osteogenetic differentiation of human mesenchymal stem cells,” *Mater. Sci. Eng. C*, vol. 32, no. 7, pp. 1926–1930, 2012.

[97] Y. Liu, T. He, and C. Gao, “Surface modification of poly(ethylene terephthalate) via hydrolysis and layer-by-layer assembly of chitosan and chondroitin sulfate to construct cytocompatible layer for human endothelial cells,” *Colloids Surfaces B Biointerfaces*, vol. 46, no. 2, pp. 117–126, 2005.

[98] F. Maccari, F. Galeotti, and N. Volpi, “Isolation and structural characterization of chondroitin sulfate from bony fishes,” vol. 129, pp. 143–147, 2015.

[99] T. Mikami and H. Kitagawa, “Biosynthesis and function of chondroitin sulfate,” *Biochim. Biophys. Acta - Gen. Subj.*, vol. 1830, no. 10, pp. 4719–4733, 2013.

[100] S. Mizumoto, S. Ikegawa, and K. Sugahara, “Human genetic disorders caused by mutations in genes encoding biosynthetic enzymes for sulfated glycosaminoglycans,” *J. Biol. Chem.*, vol. 288, no. 16, pp. 10953–10961, 2013.

[101] Jung F.; Braune S.; Lendlein A. Hemocompatibility testing of biomaterials using human platelets. Clinical *Hemorheology and Microcirculation* 2003, *53*, 97-115.

[102] Anderson J.M.; Rodriguez A.; Chang D.T. Foreign body reaction to biomaterials. *Semin Immunol* 2008, *20(2)*, 86-100.

[103] Horbett T.A. Adsorbed Proteis on Biomaterials. In *Biomaterials Science, An Introduction to Materials in Medicine*, 3rd ed.; Ratner B.D., Hoffman A.S., Schoen F.J. Lemons J.E. Eds. Elsevier INC., UK 2013 pp. 394-408.

[104] Courtney J.M.; Lamba N.M.K.; Sundaram S.; Forbes C.D. Biomaterials for blood-contacting applications. Biomaterials 1994, 15, 737-744.

[105] Ikada Y. Surface modifications of polymers for medical applications. Biomaterials 1994, 15, 725-736.

[106] Faxalv L.; Ekblad T.; Liedberg B.; Lindahl T.L. Blood compatibility of photografted hydrogel coatings. Acta Biomater 2010, 6, 2599-2608.

[107] Cashman J.D.; Kennah E.; Shuto A.; Winternitz C.; Springate C.M.K. Fucoidan film safely inhibits surgical adhesions in a rat model. J Surg Res 2011, 171, 495-503.

[108] Nair L.S.; Laurencin C.T. Biodegradable polymers as biomaterials. Prog. Polym. Sci. 2007, 32, 762-798.

[109] Azevedo T.C.G.; Bezerra M.E.B.; Santos M.D.G.D.L.; Souza L.A.; Marques C.T.; Benevides N.M.B.; Leite E.L. Heparinoids algal and their anticoagulant, hemorrhagic activities and platelet aggregation. Biomed Pharmacother 2009, 63, 477–483.

[110] Dore C.M.P.G.; , Alves M.G.C.F.; Will L.S.E.P.; Costa T.G.; Sabry D.A.; Rego L.A.R.S.; Accardo C.M.; Rocha H.A.O.; Filgueira L.G.A.; Leite E.L. A sulfated polysaccharide, fucans, isolated from brown algae Sargassum vulgare with anticoagulant, antithrombotic, antioxidant and anti-inflammatory effects. Carbohydr Polym. 2013, 91, 467–475.

[111] Hu Y.; Li S.; Li J.; Ye X.; Ding T., Liu D.; Chen J.; Ge Z.; Chen S. Identification of a highly sulfated fucoidan from sea cucumber Pearsonothuria graeffei with well-repeated tetrasaccharides units. Carbohydr Polym. 2015, 134, 808–816.

[112] Jin W.; Zhang Q.; Wang J.; Zhang W. A comparative study of the anticoagulant activities of eleven fucoidans. Carbohydr Polym. 2013, 91, 1–6.

[113] Yang Q.; Yang R.; Li M.; Liang X.; Elmada Z.C. Effects of dietary fucoidan on the blood constituents, anti-oxidation and innate immunity of juvenile yellow catfisch (Pelteobagrus fulvidraco). Fish Shelfish Immunol. 2014, 41, 264-270.

[114] Vesel A.; Mozetic M.; Strnad S. Improvement of adhesion of fucoidan on polyethylene terephthalate surface using gas plasma treatments. Vacuum 2011, 85, 1083–1086.

[115] Pielesz A.; Binias W. Cellulose acetate membrane electrophoresis and FTIR spectroscopy as methods of identifying a fucoidan in Fucus vesiculosus Linnaeus. Carbohydr Res. 2010, 345, 2676–2682.

[116] Rabanal M.; Ponce N.M.; Navarro D.; Gomez R.M.; Stortz C. The system of fucoidans from the brown seaweed Dictyota dichotoma: Chemical analysis and antiviral activity. Carbohydr Polym. 2014, 101, 804–811.

[117] Zhao X.; Dong S.; Wang J.; Li F.; Chen A.; Li B. A comparative study of antithrombotic and antiplatelet activities of different fucoidans from Laminaria japonica. Thromb Res. 2012, 129, 771–778.

[118] Ale M.T.; Maruyama H.; Tamauchi H.; Mikkelsen J.D.; Meyer A.S. Fucoidan from Sargassum sp. and Fucus vesiculosus reduces cell viability of lung carcinoma and melanoma cells in vitro and activates natural killer cells in mice in vivo. Int J Biol Macromol. 2011, 49, 331–336.

[119] Ustyuzhanina N.E.; Bilan M.I.; Gerbst A.G.; Ushakova N.A.; Tsvetkova E.A.; Dmitrenok A.S.; Usov A.I.; Nifantiev N.E. Anticoagulant and antithrombotic activities of modified xylofucan sulfate from the brown alga Punctaria plantaginea. Carbohydr Polym. 2016, 136, 826–833.

[120] Zhu Z.; Zhang Q.; Chen L.; Ren S.; Xu P.; Tang Y. Luo D. Higher specificity of the activity of low molecular weight fucoidan for thrombin-induced platelet aggregation. Thromb Res. 2010, 125, 419–426.

[121] Durig J.; Bruhn T.; Zurborn K.H.; Gutensohn K.; Bruhn H.D.; Beress L. Anticoagulant fucoidan fractions from fucus vesiculosus induce platelet activation in vitro. Thrombosis Research 1997, 85, 479-491.

[122] Fitton J.H. Therapies from fucoidan; multifunctional marine polymers. Mar Drugs. 2011, 9, 1731–1760.

[123] Friedrich J. Polymer surface modification with monosort functional groups. In The Plasma Chemistry of Polymer Surfaces: Advanced Techniques for Surface Design. Wiley-VCH, Germany, 2012, pp. 249-302.

[124] D. M. Svirachev and N. a Tabaliov, “Plasma Treatment of Polymer Surfaces in Different Gases,” *Bulg. J. Phys.*, vol. 32, pp. 22–33, 2005.

[125] P. Mohr, B. Taylor, and D. Newell, “CODATA recommended values of the fundamental physical constants: 2010,” *Rev. Mod. Phys.*, vol. 84, no. 4, pp. 1527–1605, 2012.

[126] A. Schrand, “Polymer Sample Preparation for Electron Microscopy,” *Microsc. Microanal.*, vol. 11, no. S02, pp. 702–703, 2005.

[127] D. Y. Duygu, T. Baykal, I. Acikgoz, and K. Yildiz, “REVIEW Fourier Transform Infrared (FT-IR) Spectroscopy for Biological Studies,” Gazi Univ. J. Sci., vol. 22, no. 3, pp. 117–121, 2009.

[128] F. M. Atitar, H. Belhadj, R. Dillert, and D. W. Bahnemann, “The Relevance of ATR-FTIR Spectroscopy in Semiconductor Photocatalysis,” Emerg. Pollut. Environ. - Curr. Furth. Implic., 2015.

[129] B. H. Stuart, Infrared Spectroscopy: Fundamentals and Applications, vol. 8. 2004.

[130] K.C. Hazen, D.L. Brawner, M.H. Riesselman, M.A. Jutila, J.E. Cutler, Infect Immun. 59 (3) (1991) 907-912.

[131] J.L. Garcia, P. Humpolicek, M.Lehocky, I. Junkar, M. Mozetic, Materials and technology 47(4) (2013) 473-479.

[132] Z. Adamczyk, L. Szyk-Warszynska, M. Zembala, M. Lehocky,Colloids Surfaces A Physicochem Eng Asp. 235 (2004) 65-72.

[133] Humpolicek P.; Kucekova Z.; Kasparkova V.; Pelkova J.; Modic M.; Junkar I.; Trchova M.; Bober P.; Stejskal J.; Lehocky M. Blood coagulation and platelet adhesion on polyaniline films. Colloids Surfaces B Biointerfaces 2015, 133, 278–285.

[134] Wijesinghe W.A.J.P.; Jeon Y.J. Biological activities and potential industrial applications of fucose rich sulfated polysaccharides and fucoidans isolated from brown seaweeds: A review. Carbohydr Polym. 2012, 88, 13–20.

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Kadir Ozaltin, Marián Lehocký *, Zdenka Kuceková, Petr Humpolíček, Petr Sáha, A novel multistep method for chondroitin sulphate immobilization and its interaction with fibroblast cells, Mater Sci Eng C, 2017, 70(94-100).

K. Ozaltin, M. Lehocky, P. Humpolicek, J. Pelkova, P. Saha, A New Route of Fucoidan Immobilization on Low Density Polyethylene and Its Blood Compatibility and Anticoagulation Activity, Int. J. Mol. Sci, 2016, 17(6),908

K. Ozaltin, M. Lehocky, P. Humpolicek, J. Pelkova, P. Saha, In vitro anticoagulation activity of Fucoidan from Fucus vesiculosus coated low density polyethylene, Plastko 2016, 2016, 309-315, ISBN: 978-80-7454-590-0

A. Panigrahi, B. Sulkowski, T. Waitz, K. Ozaltin, W. Chrominski, A. Pukenas, J. Horky, M. Lewandowska, W.Skrotzki, M. Zehetbauer, Mechanical properties, structural and texture evolution of biocompatible Ti45Nb alloy processed by severe plastic deformation, J Mech Behav Biomed Mater, 2016, 62(93-105)

K. Ozaltin, W. Chrominski, M. Kulczyk, A. Panigrahi, J. Horky, M. Zehetbauer, M. Lewandowska, Enhancement of mechanical properties of biocompatible Ti45Nb alloy by hydrostatic extrusion, J Mater Sci, 2014, 49:6930-6936

B. Sulkowski, A. Panigrahi, K. Ozaltin, M. Lewandowska, B. Mikuowski, M. Zehetbauer, Evolution of strength and structure during SPD processing of Ti45Nb alloys:experiments and simulations, J Mater Sci, DOI 10.1007/s10853-014-8320-2

K. Hajizadeh, H. Maleki-Ghaleh, E. Aghaie, S. Ghobadi Alamdari, M.G. Hosseini, M.H. Fathi, K. Ozaltin, K.J. Kurzydlowski, effect of equal channel angular pressing (ECAP) process on the corrosion behavior of 316L stainless steel in Ringer's solution, Corrosion, 2014, 71(3):367-375

K. Ozaltin, F. Bozduman, T. Aktan, L. Oksuz, G. Tinaz, Plasma water treatment by electrical discharge methods, 2011 IEEE International Conference on Plasma Science, DOI: 10.1109/PLASMA.2011.5993397

T. Aktan, A. Gulec, K. Ozaltin, L. Oksuz, S. Ulusoy, Atmospheric pressure plasma jet e_ects on sterilization of e. coli and s. aureus, 2011 IEEE International Conference on Plasma Science, DOI: 10.1109/PLASMA.2011.5992935

L. Oksuz, A. Gulec, K. Ozaltin, K. Akkaya, G. Erkmen, A. Uygun, Electronical and optical characteristics of atmospheric pressure plasma enhanced chemical vapor deposition (APPECVD) system, 2008 IEEE 35th International Conference on Plasma Science, DOI: 10.1109/PLASMA.2008.4590732