UNIVERSIDADE DE TRÁS-OS-MONTES E ALTO DOURO

BIOINSPIRED DEGRADABLE SUBSTRATES WITH EXTREME WETTABILITY PROPERTIES – *in vivo* study of standard and superhydrophobic poly(L-lactic acid) films

DISSERTAÇÃO DE MESTRADO EM MEDICINA VETERINÁRIA

NICOLE LOUISE LÂNGARO AMARAL

Orientadora:

Professora Doutora Maria Isabel Ribeiro Dias

Universidade de Trás-Os-Montes e Alto Douro

Coorientador:

Professor Doutor Carlos Alberto Antunes Viegas

Universidade de Trás-Os-Montes e Alto Douro

Vila Real, 2017

UNIVERSIDADE DE TRÁS-OS-MONTES E ALTO DOURO

BIOINSPIRED DEGRADABLE SUBSTRATES WITH EXTREME WETTABILITY PROPERTIES – *in vivo* study of standard and superhydrophobic poly(L-lactic acid) films

DISSERTAÇÃO DE MESTRADO EM MEDICINA VETERINÁRIA

NICOLE LOUISE LÂNGARO AMARAL

Orientadora:

Professora Doutora Maria Isabel Ribeiro Dias

Universidade de Trás-Os-Montes e Alto Douro

Coorientador:

Professor Doutor Carlos Alberto Antunes Viegas

Universidade de Trás-Os-Montes e Alto Douro

Composição do júri:

Professor Doutor Luis Maltez da Costa

Professora Doutora Maria dos Anjos Pires

Vila Real, 2017

DECLARAÇÃO

NOME: NICOLE LOUISE LÂNGARO AMARAL

TELEMÓVEL: (+351) 934704698

CORREIO ELECTRÓNICO: NICKA.LOUISE@GMAIL.COM

DESIGNAÇÃO DO MESTRADO: MESTRADO INTEGRADO EM MEDICINA VETERINÁRIA

TÍTULO DA DISSERTAÇÃO DE MESTRADO EM MEDICINA VETERINÁRIA:

BIOINSPIRED DEGRADABLE SUBSTRATES WITH EXTREME WETTABILITY PROPERTIES – *in vivo* study of standard and superhydrophobic poly(l-lactic acid) films

ORIENTADORES:

PROFESSORA DOUTORA MARIA ISABEL RIBEIRO DIAS

PROFESSOR DOUTOR CARLOS ALBERTO ANTUNES VIEGAS

ANO DE CONCLUSÃO: 2017

DECLARO QUE ESTA DISSERTAÇÃO DE MESTRADO É RESULTADO DA MINHA PESQUISA E TRABALHO PESSOAL E DAS ORIENTAÇÕES DOS MEUS SUPERVISORES. O SEU CONTEÚDO É ORIGINAL E TODAS AS FONTES CONSULTADAS ESTÃO DEVIDAMENTE MENCIONADAS NO TEXTO E NA BIBLIOGRAFIA FINAL. DECLARO AINDA QUE ESTE TRABALHO NÃO FOI APRESENTADO EM NENHUMA OUTRA INSTITUIÇÃO.

VILA REAL, 06 DE DEZEMBRO DE 2017

NICOLE LOUISE LÂNGARO AMARAL

Dissertação apresentada à Escola de Ciências Agrárias e Veterinárias da Universidade de Trás-os-Montes e Alto Douro, como requisito para a obtenção do título de Mestre em Medicina Veterinária.

ACKNOWLEDGMENTS

First of all, I want to acknowledge all my family for teachings and inspiration to follow this amazing profession. Thanks for the example of ethics, persistence, and courage, for encouraging me to follow pathways that make me competent to practice veterinary medicine, for the love, strength, patience and for understanding my absence during this time far away.

I also thank my friend at heart and colleague, Shayra Bonatelli, who always listened to me, supported me and encouraged me, even living far away so many years.

My special thanks are directed to my professor, Maria Isabel Dias, for the opportunity to carry out this work, for the companionship and support during the difficult moments when I was alone in Portugal, besides her teachings during the period, helping in my professional qualification, and for presenting me to exceptional people in the research areas.

Professor Carlos Viegas, thank you for the guidance in the preparation of the master's dissertation, for the dedication and concern to always provide quality on teaching.

Thanks also to the animals that, unintentionally, provided me the learning during the course and realization of this research.

To the Department of Veterinary Sciences of the University of Trás-os-Montes and Alto Douro, for the global support that made feasible the experimentation. To Professor António Manuel Silvério Cabrita of the Faculty of Medicine of the University of Coimbra, for making time and space available for histopathological study. To Professor João Mano and PhD Mariana B. Oliveira for performing the preparation of the biomaterials and for the orientation about the research carried out with them.

To all of you, thank you very much!

ABSTRACT

The biomaterials stimulate inflammatory responses after *in vivo* implantation and their surface wettability is known to have great influence on this aspect. It has been shown that PLLA is widely used for medical devices with rare cases of complications. However, the impact of extreme wettability surfaces on the cells behavior remain not completely understood. We evaluated the inflammatory response *in vivo* of PLLA with different wettabilities after subcutaneous implantation in rats. The materials were implanted in a total of 18 rats divided into two groups: control group (PLLA standard/hydrophobic) and experimental group (PLLA superhydrophobic). For each group, three animals (n=3) were euthanized on day 7, 14 or 60 and histological cuts of the surrounding tissue of the implants were analyzed with hematoxylin and eosin (HE) and Masson's trichrome (TM). A minimal to moderate inflammatory response was observed for PLLA superhydrophobic surface along the time and a mild to moderate for PLLA standard. At the day-7, the inflammatory reaction was classified as moderate reactive for both biomaterials and at the day-14 and -60, there were only scant inflammatory cells surrounding the implant. A reduction of the inflammatory process was verified after 60 days in comparison to 7 days for both groups, better seen in the PLLA superhydrophobic group. The TM staining showed the formation of a fibrous capsule surrounding both materials at all the intervals. The fibrous capsule at the day-7 was not well-organized, with a minimal and loose arrangement of collagen and many inflammatory cells between fibroblasts. At day-60 the capsule was well-organized containing densely packed collagen fibers, several fibroblasts and few inflammatory cells. The capsule thickness measurement revealed statistically difference along the time only in the PLLA standard group. At day-60, the capsules were thicker, with more densely arranged collagenous tissue compared with those at day-7. No difference was found for the capsule thickness related with the type of biomaterial implanted. We demonstrated good biocompatibility for hydrophobic and superhydrophobic PLLA, with no signs of severe inflammation. There was a well-ordered host response with wound healing signs and the inflammatory response decreased along the time.

Keywords: tissue engineering, superhydrophobic, biomaterial, biocompatibility, inflammatory response, wettability.

RESUMO

Quando implantados *in vivo*, os biomateriais estimulam respostas inflamatórias e a sua molhabilidade da superfície pode ter grande influência sobre este aspecto. O poli(L-ácido láctico) (PLLA) é amplamente utilizado para dispositivos médicos com raros casos de complicações. Porém, o impacto dos valores extremos de molhabilidade da superfície no comportamento das células não está completamente elucidado. Avaliamos a resposta inflamatória *in vivo* do PLLA com diferentes valores de molhabilidade após implantação subcutânea em ratos. Os materiais foram implantados em 18 ratos divididos em dois grupos: grupo controle (PLLA padrão/hidrofóbico) e grupo experimental (PLLA superhidrofóbico). Para cada grupo, três animais (n = 3) foram eutanasiados no dia 7, 14 ou 60 e os cortes histológicos do tecido circundante aos implantes foram analisados com as colorações Hematoxilina e Eosina (HE) e Tricrômio de Masson (TM). Observou-se uma resposta inflamatória de mínima a moderada para o PLLA superhidrofóbico e leve a moderada para o PLLA padrão ao longo do tempo. No dia 7, foi classificada como moderadamente reativa para ambos os biomateriais e nos dias 14 e 60 notou-se apenas poucas células inflamatórias rodeando o implante. Houve redução do processo inflamatório após 60 dias de implantação em comparação com o dia-7 para ambos os grupos. A coloração de TM mostrou uma cápsula fibrosa envolvendo tanto o PLLA padrão quanto o superhidrofóbico em todos os intervalos de tempo. A cápsula fibrosa no dia 7 era pouco organizada, com um arranjo mínimo e solto de colágeno, e muitas células inflamatórias entre fibroblastos. No dia 60, a cápsula estava bem organizada, contendo densas fibras de colágeno, vários fibroblastos e poucas células inflamatórias. A medida da espessura da cápsula revelou diferença estatística ao longo do tempo apenas no grupo padrão PLLA. No dia 60, as cápsulas eram mais espessas, com colágeno mais densamente disposto, em comparação com o dia 7. Não foi encontrada diferença na espessura da cápsula relacionada ao tipo de biomaterial. Demonstrou-se boa biocompatibilidade para o PLLA hidrofóbico e superhidrofóbico, sem sinais de inflamação grave, com sinais de cicatrização das feridas e diminuição da resposta inflamatória ao longo do tempo.

Palavras-chave: engenharia de tecidos, superhidrofobicidade, biomateriais, biocompatibilidade, resposta inflamatória, molhabilidade.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS AND ACRONYMS

CA – contact angle

ECM – extracellular matrix

FBGCs – foreign body giant cells

FDA - Food and Drug Administration

HE – hematoxylin and eosin

IgG - immunoglobulin G

Mw – molecular weight

PMN – polymorphonuclear

PLLA – poly(L-lactic acid)

PDLA – poly(D-lactic acid)

PDLLA – poly(D,L-lactic acid)

PLGA - poly(lactide-*co*-glycolide acid)

PLA – poly(lactic acid)

PGA – Poly(glycolic acid)

RM – regenerative medicine

SEM – standard error mean

TE – tissue engineering

TERM – tissue engineering and regenerative medicine

TM – Masson's trichome

UV - ultraviolet

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1. INTRODUCTION

1.1 TISSUE ENGINEERING AND REGENERATIVE MEDICINE

Over the past years, the Regenerative Medicine (RM) approaches have been studied for many reasons. The injury or failure of an organ or tissue is one of the most frequent, devastating and costly problem in human health care [1]. Reviewing the lack of consensus about a clear and precise definition of RM, Daar and Greenwood (2007) defined it as "an interdisciplinary field of research and clinical applications focused on the repair, replacement or regeneration of cells, tissues or organs to restore impaired function resulting from any cause, including congenital defects, disease, trauma and ageing" [2].

Traditionally, RM uses a combination of several technologies that stimulates and supports the body's own self-healing ability. It may include, but is not limited to, the use of soluble molecules, gene therapy, stem and progenitor cells therapy [2], immunomodulation, nanomedicine and Tissue Engineering (TE) [3]. The TE strategies stand on three main pillars: cells, scaffolds and bioactive molecules, often combined into complex systems (Figure 1) where scaffolds are typically seeded with cells and/or growth factors and give the mechanical support to cell growth and proliferation, acting as a temporal template for tissue formation [3, 4]. These mainstream components can be cultured in bioreactor systems [5] that provide biochemical and physical regulatory signals under a closely monitored and tightly controlled environment (*in vitro)* to encourage the cells to undergo differentiation and/or to produce extracellular matrix (ECM), prior to implantation into the patient [6]. The studies around RM and TE in the last years made clear that these two subjects are extremely related because of their similar objectives. Correspondingly, these two fields have been merging in nowadays as a single research pursuit, originating the wide-ranging field of Tissue Engineering and Regenerative Medicine (TERM) [3].

The progress in the field of TERM has caused a revolution in present and future trends of medicine and surgery [1, 5] and on the development of off-the-shelf tissue-engineered products, holding the potential to manage wide range of diseases, pathological conditions and traumas in the upcoming years [4]. In fact, advanced TERM approaches bring new therapeutic options for all human population, where bioengineered materials are able to improve patient outcomes and recovery time [4, 5]. In veterinary medicine, some species have been studied with the same purpose. The therapeutic options for animals include wound healing, bone regeneration, drug and vaccine delivery [7]. For the athlete horses, for example, the RM therapies based on the use of growth factors and cells aim to improve the quality and speed of

healing for faster returning to the competitions [8]. Scaffolds for the repair and reconstruction of dermatologic, musculotendinous and urogenital structures are also used for veterinary applications in other species [9].

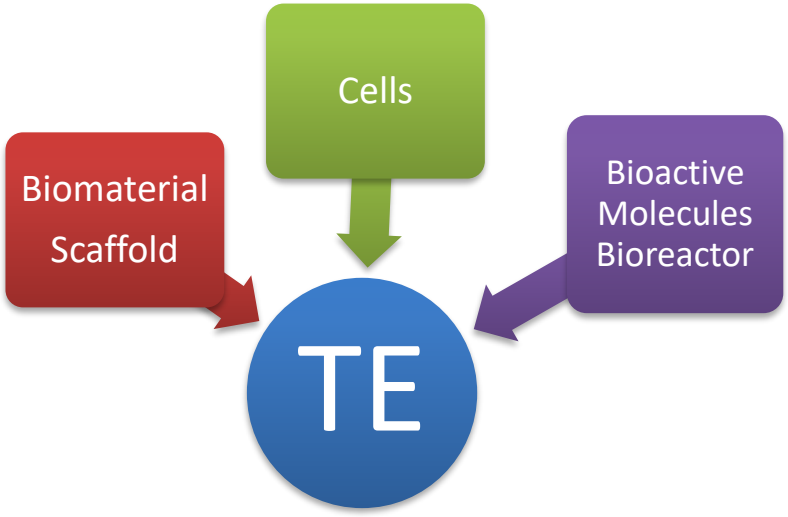

Figure 1 – TE triad of cells, chemical (bioactive molecules, e.g., growth factors) and physical (bioreactor) signals, and the scaffold which allows cells to migrate, adhere and produce new tissue. Adapted from [10].

Langer and Vacanti (1993) describe the high economic costs and the problems with organ transplantation and reconstructive surgeries. They suggest that TE can enable future savings with human healthcare by providing alternatives cheaper than donor organs and means of intervention before patients are critically ill [1]. Moreover, the engineering of cells, tissues, and organs in an external controlled environment before surgical transplantation can significantly reduce the complications of donor site morbidity [11, 12] and immunologic rejection [11, 13], consequently decreasing health care costs related to ineffective or inadequate approaches [4].

The success of TERM technologies demands deep investigation of the biological mechanisms responsible for the repairing process against the injuries, as well as the knowledge concerning the new biomaterials under consideration [5].

1.1.1 BIOMATERIALS FOR TISSUE ENGINEERING APPLICATIONS

Biomaterials for TE purposes must follow rigorous criteria and requirements to be accepted by regulatory agencies for being manufactured for clinical use [14]. In this sense, biocompatibility of the scaffold/matrix components (e.g., source, purity, and contaminates), physical properties (e.g., mass, volume, density, and porosity), degradation kinetics and sterility are essential aspects related to the safety and performance of TERM products that should be considered [15].

Materials used in biomedical applications must be nontoxic, biocompatible, and suitable for the specific application which have been designated for. It means that biomechanical properties and physical structure must be appropriated [16]. They should not contain impurities, initiators, additives, stabilizers, emulsifiers or coloring leachable that would cause *in vivo* reactions [14].

Table 1 – List of important aspects to be considered in developing biomaterials for clinical and commercial use. Adapted from [17].

Biocompatibility	**Biofunctionality (requisites related to effective use)**
Nontoxic (biosafe)	Adequate properties
Nonimmunogenic	mechanical
Noncarcinogenic	physical
Nonthrombogenic	chemical
	thermal
	biological
	Appropriate rate of degradation
	Resistance to sterilization
	Resistance to aging on storage
	Easy use
	Approved by regulatory agencies

To avoid graft failure, the biomaterial must maintain its biostability and biofunctionality during the expected implant life to ensure the function of the organ or tissue. In other words, the mechanical, chemical and structural properties for long-term use must not change over the time [14].

Considering their biostatibility, biomaterials can be classified as biostable/bioinert, bioabsorbable, and bioactive. The biostable materials, such as metals, ceramics and glasses, are projected to stay in a body the whole lifetime of the patient, functioning appropriately and causing minimal response in the surrounding tissues [16]. A common feature of these

biomaterials is that after implantation a layer of diverse unspecific proteins is adsorbed on their surface, attracting cells to grow and form fibrous tissue that will completely encapsulate the implant with time. Biodegradables are applicable to those medical devices with ability to undergo a progressive degradation while new tissue regenerates and heals [18] and ideally stay in the body only temporarily, while serving the pre-designed function, disappearing without the necessity of a second surgical intervention to remove them [19]. Another desirable feature is that they do not interfere on imaging diagnosis after being resorbed, which facilitates subsequent medical evaluations [20]. Bioactive materials, in other hand, are capable of stimulating the surrounding tissue with the aim of leading or activating the cells to specific responses and behaviors and enhance tissue growth [16, 18]. The stability features can be combined to obtain optimized materials exhibiting tailored mechanical properties and controllable degradation rates in the body [21, 22].

The choice between a material requiring long-term stability or one bioresorbable depends of its application, the organ function to be repaired, and the time of implantation that is desired [14]. Several biomaterials for TERM applications are designed to degrade or resorb *in vivo* during the tissue regeneration. Synthetic polymers such as poly(glycolide/L-lactide) and polydioxanone, or natural materials such as collagen and hyaluronic acid are commonly used for this purpose [15]. In this sense, bioabsorbable polymers are preferred candidates for developing therapeutic devices such as temporary prostheses, three-dimensional porous structures as scaffolds for TE and as controlled/sustained release drug delivery vehicles [23], since they are able to be broken down and excreted or resorbed without requiring a second surgery, reducing medical costs and other inconveniences for the patients [20, 24].

Clinically, it is desirable that materials have a predictable bioabsorption profile, because the recovery speed of the damaged tissue and the mechanical properties of the tissues may be different along the body [25]. In 2013, Willbold et al. demonstrated a correlation between the deterioration of a biodegradable metal and the site of implantation. The corrosion rate in subcutaneous was the fastest, followed by intramuscular and bony implantation of the samples. The reason for this behavior in different anatomical locations could be explained by the local blood flow that was higher in the subcutaneous site [26]. Artzi et al (2011) also analyzed materials implanted in different target sites, namely subcutaneous, intramuscular and intraperitoneal spaces. The distinctive erosion profiles in this study were correlated with the fluid volume in each site [27].

The first-generation biomaterials were designed initially to achieve adequate mechanical proprieties, such as strength, and a relative state of "bioinertness", with a minimal toxic response of the host [16]. Nowadays, with the improvements in the technologies approaches, the surface design is projected to be able to direct the surrounding biological processes to attain a desired response depending on the specific application [28]. In other words, the ideal biomaterial should recapitulate the form and activity of the ECM that supports the seeded cells *in vivo* [11], promoting their differentiation and proliferation towards the formation of a new tissue [29]. The combination of bioactivity and biodegradability is probably the most relevant characteristics in the new biomaterials that are able to stimulate particular cellular responses at the molecular level [18]. In the past decade, it has become increasingly apparent that these behaviors of biomaterials play a fundamental role in the viability and functionality of cells, tissues, and organs [11].

Generally, the classification of biomaterials fall into one of three categories: (1) naturally derived materials such as collagen or hyaluronic acid, proteins, peptides or carbohydrates, (2) synthetic polymers such as polymeric (e.g. polyglycolic acid and polylactic acid) or inorganic materials (e.g. ceramic and metals), or (3) processed tissue derived from human or animal sources, as decellularized tissue matrices obtained via treatment with a detergent [11, 30, 31]. The most commonly materials used in clinical applications are natural and modified natural materials, but also metals, ceramics, synthetic polymers, and composites [16].

Polymers are long-chain molecules that consist of a number of small repeating units [32]. They possess significant potential since flexibility in chemistry gives rise to materials with great physical and mechanical property diversity [24]. Polymers are relatively weak and ductile compared to inorganic materials such ceramics, glasses, and metals, however, due to their versatility, easiness of processing, and biocompatibility, many of them are widely and effectively used for replacement, support, augmentation, or fixation of living tissues [16]. Moreover, polymers can be prepared in different compositions with a wide diversity of structures and properties that other materials cannot [33]. Among them, the natural ones are really attractive options, mainly due to their similarities with the ECM and good biological performance [29, 34]. Natural and synthetic polymers can be combined resulting in new materials with appropriate mechanical properties of the synthetic component and biocompatibility of the biological component [35].

The biodegradable polymers have basically two major applications; as biomedical polymers for health care and as environment-friendly polymers that do not exert adverse effects on animals and plants on the earth [36].

Some of the current applications of biodegradable polymeric materials in the surgery and pharmacology include: temporary prostheses, drug delivery and targeting systems, and medicated prostheses [17]. The use of biomaterials to deliver biologically active agents directly to the site of disease in a controlled manner, sparing off-target tissue toxicities, is an interesting concept to facilitate localized therapies such as tumors [37] and periodontal diseases [38], for example. Biomaterials can also be used as scaffolds for cell transplantation; as barriers at the cellular or the protein level to guide tissue regeneration; as tissue adhesives or structural supports to bear mechanical loads during healing or regeneration; and as provisional matrices [39].

The main groups of polymeric materials used in biomedical applications and some examples of each group can be summarized as follows (Table 2).

Table 2 - Representative list of some polymers used in TERM. Adapted from [40].

Classification	**Polymers**
Natural polymers	Collagen, albumin, gelatin, agarose, alginate, carrageenan, hyaluronic acid, dextran, chitosan.
Synthetic polymers	
Biodegradable	Poly(lactic acid), poly(glycolic acid), poly(hydroxyl butyrate), poly(ε-caprolactone), poly(dioxanones), poly(sebacic acid), polyamino acids, polyphosphates, polyurethanes, polyortho esters.
Non-biodegradable	Carboxymethyl cellulose, polymethacrylate, poly(methyl methacrylate), polyvinyl pyrrolidone.

In the environmental field, polymers can be used to packaging, mulching films, agricultural staples, coatings to protect seeds, chewing gums, cigarette filters, cartridge and cartridge wax [17]. Thinking about a green environment, biodegradable polymers are very attractive, but still expensive for production [36].

1.1.1.1 NATURAL MATERIALS

Naturally derived polymers are available in large quantities and usually biodegradable [33]. They offer the advantage of being similar, sometimes identical, to naturally occurring substances of ECM, avoiding the stimulation of chronic inflammation or immunological reactions, often noticed with synthetic polymers [29]. Furthermore, due this similarity to biological macromolecules, natural biomaterials are able to be designed to work efficiently at molecular, rather than macroscopic level [41].

Another interesting characteristic of natural polymers is their ability of being degraded and remodeled by cell-secreted enzymes [42], a virtual assurance that the implant will be eventually metabolized and be removed by normal metabolic processes [41].

Some natural polymers have antibacterial properties and are used as coating materials for alleviating pathogenic colonization on surfaces. The coatings are noncytotoxic and exhibit a high degree of stability under expected conditions. For example, agarose works as antibacterial coating for biomedical devices and quaternized chitosan for preventing pathogen transmission in the environment [43].

For delivery systems, they offer the advantage of being usually non-toxic, even at high concentrations, so they can readily be incorporated into oral delivery or bolus matrix delivery systems [42].

The classification of natural polymers, some examples, their properties and applications are summarized in the Table 3.

The principal disadvantage of natural polymers lies in their structural complexity that makes difficult the development of reproducible production methods [33]. The natural variability in structure of substances derived from natural sources and the chemical difference from one species to another and from one tissue to the next induces batch to batch variations [41].

Other potential problems when using a natural polymer as biomaterial include: deficiency in bulk quantity and expansive, and the variability of degradation rate from patient to patient, once it depends upon enzyme quantities [44].

Nowadays, with the advances in biotechnology, natural polymers can be synthetized by the fermentation of micro-organisms [45] or produced *in vitro* by enzymatic polymerization [46].

Table 3 - Summary of main properties and applications of some natural polymeric biomaterials. Adapted from [33].

Natural polymer	Main applications and comments
Proteins and protein-based polymers	Absorbable, biocompatible, nontoxic, naturally available, typically elastic materials used as implants and in TE.
Collagen	Absorbable sutures, sponge wound dressing, drug delivery, artificial skin, coatings to improve cellular adhesion, guided tissue regeneration in dental applications, scaffold for reconstruction of blood vessels, wound closure.
Albumin	Cell and drug microencapsulation.
Poly(amino acids)	Nontoxic, nonantigenic and biocompatible. Used as oligomeric drug carriers.
Polysaccharides and derivatives	
From vegetable sources	
Carboxymethyl cellulose	Non-biodegradable. Cell immobilization via a combination of ionotropic gelation and polyelectrolyte complex formation (e.g. with chitosan), in drug-delivery systems and dialysis membranes.
Cellulose sulphate	Component of polyelectrolyte complexes for immunoisolation.
Agarose	Largely used as supporting materials in clinical analysis and as an immobilization matrix.
Alginate (marine sources, algae)	Excellent gel-formation properties; relative biocompatibility; batch-to-batch variations. Used as immobilization matrices for cells and enzymes, controlled release of bioactive substances, injectable microcapsules for treating neurodegenerative and hormone-deficiency diseases.
Carrageenan	Excellent thermoreversible properties. Used for microencapsulation.
From human and animal sources	
Hyaluronic acid	Excellent lubricant, potential therapeutic agent.
Heparin and heparin-like glycosaminoglycan	Antithrombotic and anticoagulant properties. Extensively used in surgery.
Microbial polysaccharides	
Dextran and its derivatives	Excellent rheological properties. Plasma expander. Widely used as drug carrier.
Chitosan and its derivatives	Biocompatible, nontoxic, excellent gel- and film-forming ability. Widely used in controlled-delivery systems.

1.1.1.2 SYNTHETIC MATERIALS

Synthetic polymers offer a number of advantages for applications in TERM. Unlike natural materials, they can be easily reproduced keeping quality and purity [47] and have better mechanical and thermal stability [48]. Moreover, they can be fabricated into various shapes with desired morphologic features [49], including three-dimensional structures with a projected dimension by three-dimensional printers [50]. They are available in many compositions with readily adjusted properties by processing, copolymerization and blending, which optimize their mechanical and biological properties [33].

In the biomedicine field, synthetic polymers are often used for TE in various areas such as the cardiovascular system, orthopedics, neurology, drug delivery systems and others [51], as represented below (Table 4).

Table 4 - A summary of the main properties and applications of some synthetic polymeric biomaterials. Adapted from [33].

Synthetic polymers	Main applications and comments
Aliphatic polyesters	
Poly(lactic acid), poly(glycolic acid) and copolymers	Used in sutures, drug-delivery systems, barrier membranes, guided tissue regeneration (dental applications), orthopedic applications, stents, staples and TE. Biodegradable. Often copolymerized to regulate degradation time.
Poly(ε-caprolactone) and copolymers	Biodegradable, used as a matrix for long term drug-delivery systems, cell microencapsulation. Properties can be changed by chemical modification, copolymerization and blending.
Polyamides (nylons)	Sutures, dressing, haemofiltration membranes.
Poly(ortho esters)	Surface-eroding polymers. Application in sustained drug delivery, stents, ophthalmology.
Poly(cyano acrylates)	Biodegradable. Used as surgical adhesives and glues, potentially used in drug delivery.
Polyphosphazenes	Made into films and hydrogels. Applications in drug delivery, blood contacting devices, skeletal reconstruction.
Thermoplastic polyurethanes	Good elastomeric properties. Used in permanently implanted medical devices (prostheses, vascular grafts), catheters and drug-delivery systems. Initial candidates for the artificial heart.

Poly(glycolic acid) (PGA), poly(lactic acid) (PLA) and their copolymer [poly(lactide-*co*-glycolide)(PLGA)] are becoming the most commonly used [23 , 52] and the most widely investigated for TE purposes [18, 53]. In particular, synthetic biodegradable polymers have attracted special attention because they better control their physico-chemical properties [18] and they can be metabolized by human body [23, 52]. These polymers degrade by nonenzymatic hydrolysis and their nontoxic degradation products are eliminated from the body by natural metabolic pathways [20], in the form of carbon dioxide and water [54] (e.g.: urine) [20]. The biodegradation rate of synthetic biodegradable polymers depends of their characteristics such as shape, molecular weight, composition, monomer conversion, macromolecular orientation, etc. and can be controlled by alteration of some features, such as copolymer ratio [25]. The degradation times can be achieved from several weeks to several months [54] for applying to clinical uses.

Although the degradation products have shown to be nontoxic, the concern with the use of some specific polymers still remains because they can provoke adverse effects or alter local microenvironment *in vivo*, reducing local pH, and consequently inducing inflammatory response and injuries in the cell health at the implant site [55]. As synthetic polymers are often associated with inflammatory reactions, except for poly(ethylene oxide) and PLGA that show good biocompatibility, their use is limited to solid, unmoving, impermeable devices [33]. Other disadvantages of the synthetic polymers, such as poor processability and loss of mechanical properties very early during degradation, are also reported [49].

Among the biopolymers used in the medical field, the polyester PLA has received significant attention, not just because it is made from renewable resources, but also because it provides excellent properties at a low cost compared to other traditional biodegradable polymers used for the same purposes [56]. PLA represents one of the most important biodegradable polymers, being the preferred alternative to its homologous PGA because of its degradation time [25]. The hydrophobic characteristic of PLA makes its degradation slower than PGA. The water absorption of thin films is limited by its hydrophobicity that slows down the backbone hydrolysis rate. According to the available data, the estimate duration of PLA degradation process is one to two years [57].

Owing to mechanical, biological and thermoplastic properties, PLA is convincingly accepted for using in biomedical applications, such as bone fixation devices. It is derived from lactic acid, a naturally occurring organic acid that can be produced by fermentation [23] of sugars obtained from renewable resources such as sugarcane [20]. Additionally, PLA is

approved by the Food and Drug Administration (FDA) [49], can be produced using low energy and used in an environmentally friendly cycle [58], being considered an eco-friendly biomaterial [58, 59].

Despite the advantages, some drawbacks may limit the use of PLA in certain applications. The limitations include poor toughness and lack of reactive side-chain groups [58].

Lactic acid is a chiral molecule, existing in L and D isomers and the term "poly-lactic acid" refers to a family of polymers: pure poly(L-lactic acid) (PLLA), pure poly(D-lactic acid) (PDLA), and poly(D,L-lactic acid) (PDLLA) [52] - a racemic mixture of PLLA and PDLA (Figure 2). As far as use in biomedical research, only PLLA and PDLLA have shown promising and have been widely studied [24]. Because it is preferentially metabolized in the body, the (L) isomer of lactic acid is often chosen for most applications [49].

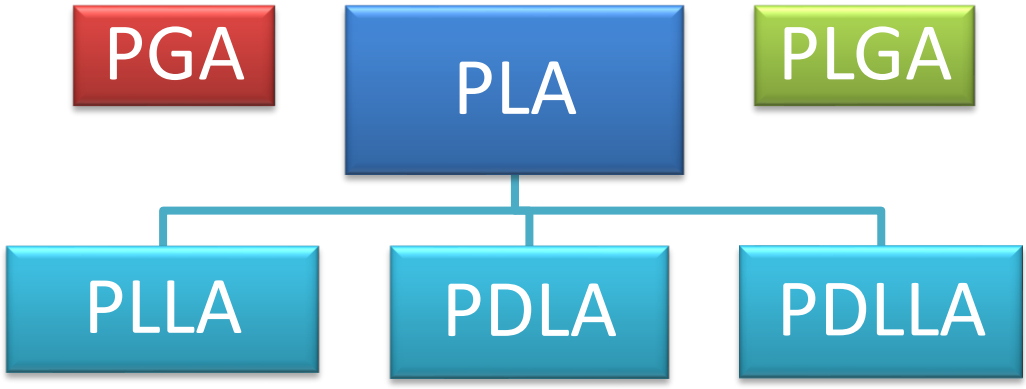

Figure 2 – PGA, PLA and their copolymer PLGA are different biomaterials used and widely investigated for TE purposes. PLA refers to a family of polymers: PLLA, PDLA and PDLLA.

PLLA is a semi-crystalline polymer [60], aliphatic polyester with good biodegradability and biocompatibility [16], versatility [61], reasonable mechanical properties, and processability in forming fibers [16]. The hydrolytic degradation process of aliphatic polyesters occurs by random scissions of ester bonds within the polymer chains [62]. Of the two enantiomeric forms (PLLA and PDLA), PLLA degrades the slowest [59], because the material has no affinity with body fluids [63]. The water uptake of PLLA during the hydrolysis is one of the responsible for the process of mass loss [64]. PLLA degradation process provides a significant increase in the crystallinity with ageing time [65], which restricts the water uptake into the polymer matrix, making the hydrolytic process difficult [64]. Consequently, some portions of biomaterial

remain protected against the water and the sorption processes lasts longer. Therefore, crystallization appears to be effective in increasing the hydrolytic stability [57].

In order to modify the degradation time to obtain a desirable time scale for specific application, investigators have blended or copolymerized PLLA with other degradable polymers [64, 66]. It offers great promise in a wide range of commodity applications, although features such as high rigidity and hydrophobicity limit its use in some areas [63].

PLLA is widely used in compounding with other materials for sutures and bone fillings [16] and also for medical devices (e.g., screws for fixation of tendon to bone and bone to bone) with rare complications [67]. However, in 1995, Bergsma et al. reported swelling at the site of implantation in four patients three years after implantation of PLLA and associated the disintegration of PLLA with it [68]. In dermatology area, Funt and Pavicic (2013) reported dermal filler complications such as granuloma in the implant site [69], but this reaction is usually attributed to inadequate techniques and not to the implant itself [70].

In summary, the success of synthetic polymers as biomaterials mainly relies on their wide-ranging mechanical properties, transformation processes that allows a variability of shapes to be easily achieved, and low production costs. On the other hand, biological polymers present good biocompatibility but their mechanical properties are usually inferior, the necessity of preserving biological properties complicates their processability, and their production or recovery costs are very high [70, 71]. “Bioartificial polymeric materials” is a term to designate a new class of materials based on blending synthetic and natural polymers, where the final purpose is produce materials for biomedical applications that possess both good mechanical properties and biocompatibility, overcoming the poor performance of each one in these features [72].

1.1.2 SUPERHYDROPHOBIC SURFACES

To meet the specifications which biomaterial was designed for, it must exhibit expected mechanical, physical, or electrical properties [14]. The good performance clinically of a biomedical device depends on the identification and controlled modification of key intrinsic surface properties [33]. Characteristics such as charge, polarity and energy, heterogeneous distribution of functional groups, wettability, chain mobility, as well as morphological and

topographical aspects, including texture, smoothness and roughness, should be considered in this sense [14].

Among the methods to characterize the wettability of the surface, the water contact angle (CA) measurement is often used [73]. According to Marmur (2012), CA is defined as the angle between the solid surface and the tangent to the liquid surface (on the liquid side of it), at the three-phase contact line. In his study, a well-defined terminology that accounts for both the chemistry of a solid surface and its wetting functionality is presented [74]. In summary, surfaces with water CAs above 90° are considered hydrophobic, and those with CAs above 150° are termed superhydrophobic (Figure 3) [75]. Moreover, the combination of suitable surface roughness and low-surface-energy materials is also responsible for superhydrophobicity [73, 76].

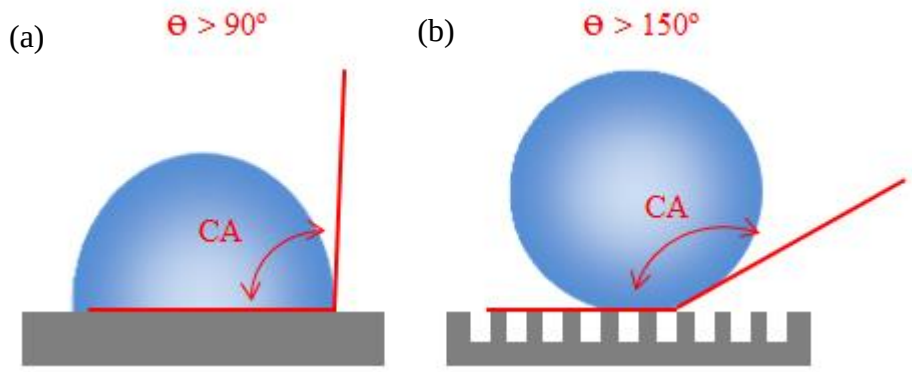

Figure 3 – Wetting on the surfaces. (a) Surfaces with water CAs above 90° are considered hydrophobic. (b) Surfaces with water CAs above 150° are termed superhydrophobic. Roughness is also responsible for superhydrophobicity. (ɵ: angle) Adapted from [77].

In the nature, we can find many superhydrophobic surfaces that possess water-repellent, self-cleaning and anti-icing properties [78]. In 1997, Barthlott and Neinhuis documented an almost complete self-cleaning ability by water-repellent plant surfaces, such as lotus leaf. In nature, many terrestrial plants and animals have the ability to create their superhydrophobic surfaces from a microscopic roughness over coated with specific functional groups [79]. Since then, the lotus leaf has become widely explored and has inspired many investigators to research others superhydrophobic plant surfaces [78] such the petal of red rose [80]. Compared with the lotus effect, which water droplets roll off the surfaces independent of their chemical nature or size providing a very effective anti-adhesive property against particulate contamination [79], the petal effect does not permit the water droplet roll off even when the petal is turned upside down [80].

Nowadays, the studies around non-wettable surfaces with high water CAs and facile sliding of drops continue to receive important attention [81] not only for academic reasons, but also for practical applications such the production of hydrogels. The drops placed onto a superhydrophobic surface almost completely surrounded by air or another desired atmosphere will maintain the spherical shape. This permits an efficient preparation of a large range of systems under mild conditions avoiding any loss of the cargo during the process and a good control over the size of the particles [82].

Hydrogels are cross-linked hydrophilic polymers of natural or synthetic origin [83] that swell significantly when placed in a polar liquid solution [84]. Hydrophilic functional groups attached to the polymer enable the hydrogels to retain high percentage water content [85], over 90% [83]. Superhydrophobic surfaces permit to encapsulate cells into hydrogels beads by gravitational dripping [82]. Hydrogels are mechanically strong and resistant to heat, wear and attack by solvents. However, they are relatively inflexible, insoluble and infusible. Most of them have applications on medical field as drug delivery and for TE [86].

Due to the limitation on fluid absorption, water-repellent devices are desirable where long-term mechanical resistance is required, as previously mentioned. For example, orthopaedic and dental implants must be water repellent to avoid any degradation or erosion processes leading to changes in toughness and loss of mechanical strength [33]. High hydrophobicity can significantly enhance the resistance against hydrolytic degradation [87] through a weak interaction between body fluid and the implanted biomaterial [63]. Others must have limited moisture penetration, as pacemakers and artificial blood vessels [33]. Sun et al. (2005) reported the effect of special nanostructures on blood compatibility. The results demonstrated excellent anti-adhesion to platelets and the relatively low platelet activation making nanostructured superhydrophobic films good candidates for utilization in artificial organ implantation, manmade blood vessels, and other blood-contacting medical devices [88].

The surface energy of an implant, indirectly measured by liquid-solid CA, also affect the biological response to the implant such as adhesion of proteins and other macromolecules onto the surface, hard and soft tissue cell interactions and bacterial adhesion and subsequent biofilm formation [89]. Two materials with similar surface energies but different water-sorption characteristics can possess different biocompatibilities [33]. However, wettability alone does not play the dominant role in determining subsequent cell behavior; the functional groups on the surfaces also affect the biomaterial performance [90]. In the study performed by Sartoretto et al. (2015), the bone healing was not affected by micro topography but by chemical changes.

Surfaces with less carbon had a markedly enhanced hydrophilicity and this accelerated osseointegration and increased the area of the bone-to-implant interface [91]. Depending on the objective of the biomaterial it should stimulate cell adhesion or suppress the attachment of specific proteins and cells [92], so wettability, surface energy and chemical property can determine the choice of a biomaterial.

It has been shown that the surface roughness of implants may also influence biological responses [93]; however surface wettability was recognized as a critical factor to explain the different cell behavior on biomaterials when comparing these two properties [60]. The cells can adhere and proliferate on both superhydrophobic and superhydrophilic surfaces, but constant contact to superhydrophobic surfaces is required for cell division and proliferation on it [94]. Modifying just one side of the surface to transform it in superhydrophobic can permit the use of this biomaterial for bone guided regeneration where this surface do not allow cell growth, for example [95].

According to Ishizaki, Saito & Takai (2010), cells easily adhere and proliferate immediately after seeding on superhydrophilic surfaces [94]. Oliveira et al. (2011) demonstrated higher cells proliferation in surfaces with water CA ranging from 13º to 30º, independently of being rough or smooth [95]. Sawase et al. (2008) studied the effect of photo-induced hydrophilicity on initial cell behavior and bone formation. The CA of the biomaterial irradiated with ultraviolet (UV) light decreased and the cell attachment and proliferation on this hydrophilic disk increased improving the initial cell reactions and enhancing early bone apposition to the implant [93].

Last studies highlighted the influence of roughness on cell behavior and protein adsorption demonstrating that total protein adsorption and cell viability at the rough surfaces are generally lower than at the corresponding smooth surfaces in superhydrophobic biomaterials [60]. However, the same authors concluded that chemistry and topography did not have the same importance to cell behavior as the wettability. Aqueous solutions in contact with superhydrophobic films have less actual surface area available for protein adsorption than the surface area of a flat surface [77] as it is demonstrated on Figure 3. On the other hand, Oliveira et al. (2011) concluded in their study that superhydrophilic surfaces seem to be ideal for repellence of proteins [95].

It has been demonstrated that increased surface roughness is also an important physical factor for bacterial adhesion [96]. In general, the surfaces with a higher number of attachment points will attract more cell attachment [97]. Bacterial adhesion shows a direct positive

correlation with the surface roughness [98-100]. It possible to take an example from dental analysis that showed an increase in plaque accumulation in rough surface above a certain threshold of roughness [101].

Analyzing the surface wettability, Tang et al. (2011) concluded that although the *Staphylococcus aureus* was not totally absent on the superhydrophobic surfaces and the amount of adhered bacteria increased with time, they were much less in quantity and more scattered than those on the hydrophilic and hydrophobic surfaces and could be easily removed. The experiment results show that superhydrophobic surfaces display high resistance to bacterial contamination and have a strong potential to reduce device-associated infection [102]. On the other hand, Sousa et al. (2011) showed that superhydrophobic and hydrophobic PLLA surfaces are able to be colonized by bacterial cells, although this effect can be due to the combined effect of the different PLLA and *S. aureus* specific surface morphologies, since *S. aureus* cells seem to fit perfectly the irregularities on the roughness of superhydrophobic surface and, thus, end up having a greater contact area than on the smooth hydrophobic surface [103].

The database of bacterial adhesion on superhydrophobic surfaces is not yet sufficiently extensive and systematic to completely understand the mechanisms of this process. With this purpose, the tests should be standardized and more bacterium types should be tested. More parameters such as surface roughness, morphology, functional group and the free energy from the superhydrophobic surfaces should also be studied to analyze their effect on bacterial adhesion [97].

It is not surprising that contradictory results have been observed in different studies, since the variability on surface evaluations and the experimental conditions applied on them make difficult to comparison about the influence on cells behavior, protein adsorption [104] and bacterial adhesion [97]. It is hard to define the true surface reactions responsible for the performance of biomaterial because several surfaces can be identical on wettability while their surface chemistries can remain quite different [90]. According to Wennerberg and Albrektsson (2009), the difficulty is occasionally attributed to the terminology assumed by the authors. In addition, many investigators falsely assume the roughness of the implant based on the surface preparation and many other studies use only qualitative techniques to define it [104].

1.1.2.1 APPLICATION OF SUPERHYDROPHOBIC SURFACES IN THE BIOMEDICAL FIELD

Various different natural, synthetic and hybrid polymers are available for biomedical applications in diverse areas. Specifically, superhydrophobic surfaces have been actively studied for the use in the industries and further in the biomedical field as substrates to control protein adsorption, cellular interaction, and bacterial growth, as well as platforms for drug delivery devices and for diagnostic tools [105].

The fact that cells adhere very differently to hydrophobic and hydrophilic substrates can be used in favor of biomedicine [92]. The different ability for proteins to adsorb in these substrates has been used to produce smart surfaces for programmed adsorption and release of proteins in the context of microfluidic devices [106].

New methodologies based on the use of superhydrophobic surfaces propose the production of compartmentalized multilayered polymeric spherical particles with controlled size and layer thicknesses of distinct materials that could allow the distribution of cells or drugs by layers and the use in a wide range of applications including cosmetics, pharmacy, agriculture, food technology and biomedicine [107]. Others recent studies propose the production of smart systems incorporating responsive substances, for example magnetic responsive particles or networks, containing temperature responsive polymers. Even growth factors or other unstable as expensive non-volatile molecule could also be integrated into such particles with high levels of efficiency [108].

Additionally, superhydrophobic surfaces with controlled wettable spots can be used as platforms to produce microarray chips for multiplexing evaluation, offering the possibility to screen individually and in the same chip different combinations of biomaterials under different conditions, including different cells, culture media or solutions with diverse proteins or other molecules [109]. These systems exhibiting patterned high-contrast wettability regions act as mini-bioreactors with distinct behaviors in each spot that may be used to distinct applications needs [110] on TERM, cellular biology, diagnosis, drug discovery and drug delivery monitoring [109]. In this sense, Ishizaki, Saito & Takai (2010) also studied the cell behavior on superhydrophobic and superhydrophilic micropatterned surfaces. The results show that the method could contribute to development of cell-based technologies including biosensors for the screening of drug libraries as well as for better understanding the eukaryotic cells interactions with implantable biomaterials and the communication between them [94].

It was recently shown that rough superhydrophobic PLLA surfaces are colonized by bacterial cells, introducing a possible application of PLLA-based superhydrophobic materials as bacterial colonization substrata with potential to be used as carriers for biomass immobilization in bio-reactors [103]. However, other study indicated that superhydrophobic surface shows high resistance to bacterial contamination and could be used in the clinical practice as antimicrobial to reduce device-associated infections [102]. This characteristic makes these materials suitable in extracorporeal medical devices, by making the device free of contamination and easy to clean [88]. For further information, researches should focus in analyzing different bacteria, regarding their Gram-type and morphology, on superhydrophobic surfaces with distinct properties.

As previously described, the excellent anti-adhesion to platelets and the relatively low platelet activation, show the useful of these films in artificial organ implantation and blood vessels, and other blood-contacting medical devices [88].

Li et al. (2013) demonstrated the application of superhydrophobic surfaces in conducting biological assays for rapid human blood typing analysis using a liquid drop micro reactor with only a small amount of blood sample. The characteristics of superhydrophobic surfaces can help the pathological laboratories on diagnosis because they enable the blood and antibody droplets to have a spherical shape, making easier to photograph, record and analyze the haemagglutination reaction inside the droplet by software [111].

Concluding, superhydrophobic surfaces can be used to produce biomaterials with a wide potential applications including catheters, endotracheal tubes, or medical instruments with a superhydrophobic coating to reduce bacterial adhesion when in contact with blood or bodily fluids; controlled patterns of superhydrophobic and superhydrophilic regions used to construct cellular microarrays or engineered tissues; disposable microfluidic diagnostic devices, where the superhydrophobic coating supports droplets or facilitates fluid flow; and coated medical devices for drug delivery [105].

Regarding the efficiency of the therapy, each application designed for a biomaterial demands appropriated characteristics such as physical, chemical, biological, biomechanical and degradation properties. For this purpose, investigators keep focus in studying a wide range of natural or synthetic polymers [112]. Superhydrophobic surfaces made by these sources have shown great potential for such applications in the biomedical field and should be better studied to improve the efficiency in the treatment of human and veterinary patients.

1.1.3 IMUNNE RESPONSE TO BIOMATERIALS

Once the inflammatory response caused by biomaterials is an unavoidable event affecting the tissue regeneration, attention to this aspect should be considered when developing TE strategies [113]. Although improvements has been made concerning about biocompatibility, many materials and procedures are associated with side effects such as inflammation, infections and subsequent loss of function [114], as well as fibrosis and thrombosis [115] inducing bioincompatibility.

In general, failure of most implants results from an inadequate host response to the material because of the organism inability to predict and regulate biological phenomena, such as protein adsorption and cell interactions [116]. Polymorphonuclear leukocytes, monocytes, macrophages and foreign body giant cells (FBGCs) play a central role in the foreign body reaction and immune inflammatory responses, processes that affect the biostability, biocompatibility and effectiveness of the implant [117].

Biocompatibility is generally defined as "the ability of a biomaterial, prosthesis, or medical device to perform with an appropriate host response in a specific application" [118] and it implies the absence of adverse reactions, local or systemic, caused by material to the tissue directly or through the release of their material constituents [119].

The ability to mimic repair processes following injury and to control reactions like inflammation has been shown to dictate the efficiency of biomaterial implants [120], as mentioned above. Then, in order to design materials with a good performance to ensure a desirable cell survival, migration and adhesion, a depth understanding of the host response is required [121].

Once implanted, the biomaterials surfaces interact with the surrounding tissues [116, 121], producing some degree of tissue damage, which will initiate two principal reactions, inflammation and the related response of repair – the wound healing [120]. In this interaction, host reactions incorporate a combination of many processes [122], summarized in the Table 5.

The response to tissue injury is dependent on multiple factors including the extent of injury, blood-material interactions and the extent of the inflammatory response that consequently will affect later events [118]. Microvascular injury, protein exudation and accumulation, and activation of the humoral and cellular defense systems are some key points occurring during the early inflammatory response [124].

Table 5 - Sequence/continuum of host responses following implantation of biomaterials. Adapted from [118, 123].

Injury post implantation
Blood/material interactions
Acute inflammation
Chronic inflammation
Granulation tissue
Foreign body reaction
Fibrosis/fibrous capsule formation

The contact between the blood and the implant is an inevitable and early occurrence after almost all implantation procedures in biological tissue [124], including soft and hard tissues (e.g. dental implants, prostheses). This initial contact with the blood influences the inflammatory reaction against the material [114] and triggers a complex series of interlinked events including protein adsorption, platelet and leukocyte activation/adhesion, and the activation of coagulation [125] and an immediate complement-mediated inflammatory response [125, 126].

Blood-biomaterial contact induce the quick adsorption of proteins onto a biomaterial surface [127], regarded as important determinant of the acute inflammatory response [128] and the first major step in the integration of an implanted device with implications for nanotechnology, biomaterials and biotechnological processes [129].

Among these host proteins that spontaneously associate with implant surfaces, albumin, immunoglobulin G (IgG) and fibrinogen usually predominate [130]. Albumin is known to decrease platelet adhesion to polymer surfaces and improve the biocompatibility, preventing the formation of thrombus [131]. IgG involves the activation of the complement system and subsequent stimulation of adherent macrophages by complement products [132]. Adsorbed fibrinogen has been shown to be the primary component of plasma responsible for acute inflammatory responses, mainly by facilitating phagocyte recruitment at implant surfaces [133]. Also, fibrinogen enhances platelet adhesion [134, 135] stimulating thrombosis [125, 136], and bacterial colonization [137].

Blood-interactions and protein adsorption on the surface of biomaterial is followed by acute inflammation, with attraction of polymorphonuclear leukocytes (PMN). Sequentially,

chronic inflammation characterized by the presence of monocytes and lymphocytes usually resolve within the first 2-3 weeks following implantation [123].

The predominant cell type present in the inflammatory response varies along the time (Figure 4) [118, 138]; however the components of the reaction within the implant site may also vary depending of the surface properties [138]. In general, neutrophils predominate during the first several days and disappear after 24 to 48 hours following injury and then are replaced by monocytes that after differentiate into macrophages which are very long-lived (up to months) [118] and the principal responsible for wound healing [138].

Implanted biomaterials usually provoke a persistence of an inflammatory stimulus leading to chronic inflammation characterized by the presence of macrophages, monocytes and particularly lymphocytes and plasma cells with the proliferation of blood vessels and connective tissue [139]. Implant failure may be caused by the fragments of implanted biomaterials that lead to chronic inflammation [128], the main reason for this undesirable outcome.

The granulation tissue process involves proliferation, maturation, and organization of endothelial cells into capillary tubes, fibroblasts proliferation and subsequently synthesis collagen and proteoglycans [140]. The sequence ultimately ends in the formation of foreign body giant cells at the tissue/material interface [121].

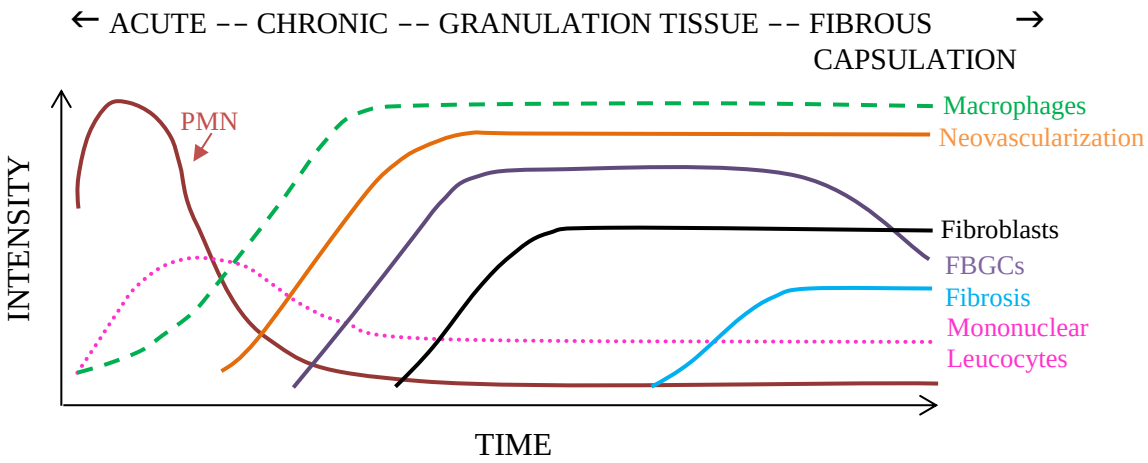

Figure 4 - The cell type temporal variation in the inflammatory response to implanted biomaterials. Adapted from [138].

The following inflammatory and wound healing response after biomaterial implantation is characterized by the foreign body reaction [121], composed of FBGCs and the components of granulation tissue described above (e.g. macrophages, fibroblasts, collagen and capillaries)

[138, 140]. Monocytes adhered to the biomaterial surface differentiate into macrophages that fuse to form the FBGCs [123] and remain at biomaterial-tissue interfaces for the lifetime of the device [122]. Maluf-Meiken et al. (2006) showed that the number of multinucleated giant cells increased significantly from the seventh day after implantation of bioabsorbable polymer, decreasing from the twenty-eighth day to the sixtieth day and increasing again from the ninetieth day [141].

The end-stage healing response to biomaterials is generally followed by fibrosis or fibrous encapsulation [140]. Mainil-Varlet, Gogolewski & Nieuwenhuis (1996) studied the tissue capsule formed around PLLA implanted in the subcutaneous tissue of sheep and noticed the capsule consisting of fibroblasts, fibrocytes, phagocytes, a few FBGCs and PMN cells. At three months post-implantation, the capsule was denser, its thickness and cellularity had slightly increased compared to one month time point and continually increased until 6 months when it showed more matured [142].

As previously described, the cell-interaction with the implant is largely dependent on the cell type and surface properties of the materials [116] such as wettability, energy, roughness, charge and chemical composition [143]. Such properties modulate the foreign body reaction in the first two to four weeks following implantation [121] and the intensity and the time variables also depend of the extent of injury created in the implantation procedure [140].

The quality of protein adhesion in the early phases of inflammation influences the cells morphology and their proliferation and differentiation ability [144]. The absence of adsorbed proteins, or interference with their function, modifies the cells behavior preventing their attachment [145]. Therefore, it is reasonable to accept that modifications in distribution of adsorbed proteins can be used in favor to engineer materials with the desired performance for a specific application [146] and with lower or no complement-activating properties [114]. Ekdahl et al. (1993) analyzed the complement activation *in vitro* and the results suggested enhancing the biocompatibility of polystyrene surfaces after surface modifications [147].

The biocompatibility of polymers is also defined by the degradation products and their active biocompatibility must be demonstrated over time. The chemical, physical, mechanical and biological properties of a biodegradable material will vary and these changes can cause long-term host responses to these biomaterials to be greatly different than the initial response [24]. In fact, degradation products of polymers may reduce the microenvironment pH and consequently affect the integrity of the cells [16]. The degradation products of PLLA, for example, reduce local pH, accelerate the polyester degradation rate and induce inflammatory

reaction [57]. Although PLA-PGA biomaterials are generally biocompatible and non-toxic, Athanasiou, Niederauer & Agrawal (1996) summarized some studies with PLA and PGA that reported inflammatory reactions usually occurring 7-20 weeks after implantation in the body [148].

Although inflammatory reaction is usually associated to implants failure, it was also reported to help the triggering of tissue regeneration (e.g. neural regeneration) [113]. Studies have been demonstrated that there is still a necessity to understand the mechanisms involved in the biocompatibility in order to improve the biomaterials and the patient's recovery, reducing undesirable reactions, treatment time and cost. For this purpose, more detailed *in vivo* studies should be performed to better explaining biomaterial-host interaction.

CHAPTER 2. EXPERIMENTAL SECTION

2.1 OBJECTIVE

With the purpose to optimize the structure of PLLA, we propose evaluate *in vivo* the biological behavior of this biomaterial in terms of its inflammatory response after subcutaneous implantation in rats. We have focus on wettability modifications of the surface, as well in morphology of PLLA to evaluate the inflammatory responses.

2.2 MATERIALS AND METHODS

2.2.1 POLY (L-LACTIC ACID) SURFACES

Biomimetic superhydrophobic surfaces were obtained from a commercially available smooth polymeric surface, PLLA of high stereoregularity (Cargill Dow Polymer Mn = 69 000, Mw/Mn = 1.734), as previously described in [149]. According to this method, a PLLA/dioxane 13% (w/w) solution was cast on this substrate and after an evaporation period of 4 minutes the substrate was immersed in ethanol during 1 hour. Dioxan was purchased from Fluka (p.a. 99.5%) and ethanol absolute from Panreac. This process induces the creation of particular structures on the surface, which will exhibit hierarchical roughness architectures. Finally, the samples were dried in a vacuum oven at 30ºC during one day to remove all the residues of solvent and nonsolvent. After, it was possible to remove the upper part of the sample easily (Figure 5). All PLLA surfaces were punched into circular samples with a diameter of 6 mm and sterilized by ethylene oxide before the transplantation.

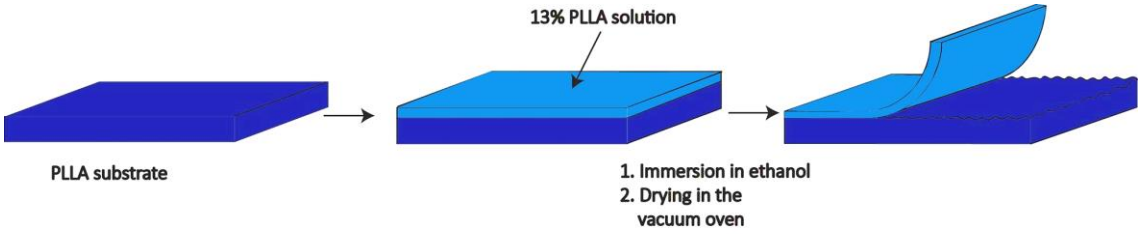

Figure 5 - Schematic representation of the experimental process to produce superhydrophobic surfaces. Adapted from [149].

2.2.2 IMPLANTATION OF THE SURFACES IN RATS

PLLA circular implants (standard and superhydrophobic), measuring approximately 6mm, were implanted in a total of 18 male *Rattus norvegicus* rats (16 weeks old), weighing between 350-400g, divided into two groups: nine animals in the control group (PLLA standard/hydrophobic) and nine animals in the experimental group (PLLA superhydrophobic). Animals were individually anesthetized with an intraperitoneal injection of medetomidine (Domitor®) (0.5 mg/kg) and ketamine (Ketamidor®) (75mg/kg) (Figure 6). The animals were immobilized and placed in a ventral position. Subsequently, the dorsal skin of the animals was shaved, washed, and disinfected with povidone-iodine. On each side of the vertebral column, four paravertebral incisions were made, two at the level of scapula and two at the level of pelvis, after, a subcutaneous pocket was created using blunt dissection with scissors and each animal was implanted with four polymer disks of the same type. After insertion of an implant, the skin was closed using skin non-absorbable suture thread. A schematic representation of the process is presented in Figure 7 and the surgical instruments utilized are shown in Figure 8. A total of 72 implants (36 implants of each group) were distributed in 18 rats (4 implants per rat). After the surgical procedure, atipamezole (Antisedan®) (1mg/kg) was administered by intraperitoneal injection with the intention of reversing the anesthesia. During the study, animals were kept in separate cages and fed with commercial rat food and water *ad libitum*. For each group, three animals (n=3) were euthanized on day 7, 14 or 60 after the wound closures and subsequently, the wounds together with their surrounding host skins were harvested for histological analysis.

Figure 6 - Drugs utilized for intraperitoneal anesthesia (Ketamidor® and Dexdomitor®) and reversal of anesthesia (Antisedan®) in the *Rattus norvegicus* rats.

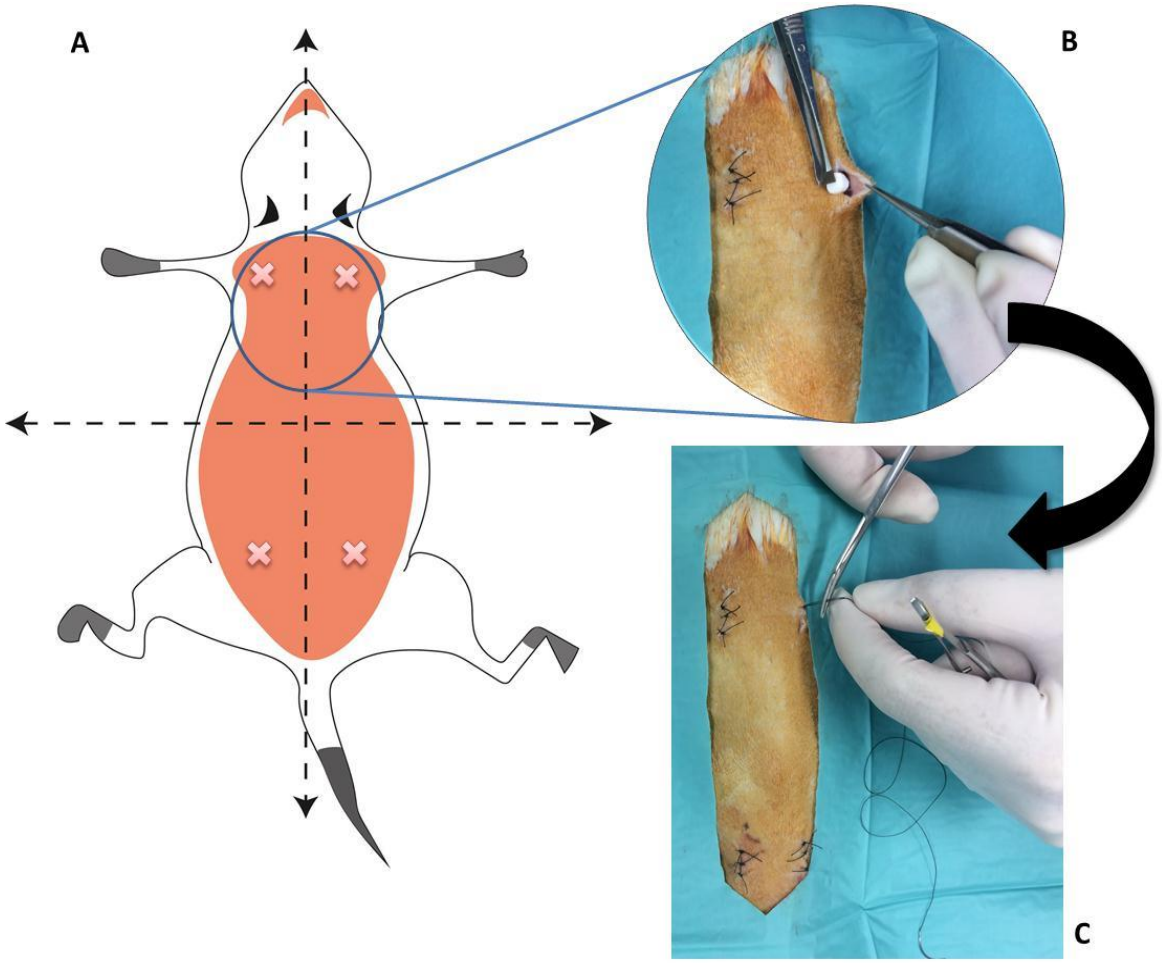

Figure 7 – Schematic representation of the process of implantation of PLLA surfaces. A) The animals were immobilized and placed in a ventral position. The dorsal skin of the animals was shaved, washed, and disinfected. Two paravertebral incisions were made at the level of scapula and two at the level of pelvis (rosy✖). B) A subcutaneous pocket was created and the implant was inserted. C) After implant insertion, the skin was closed using skin non-absorbable suture thread.

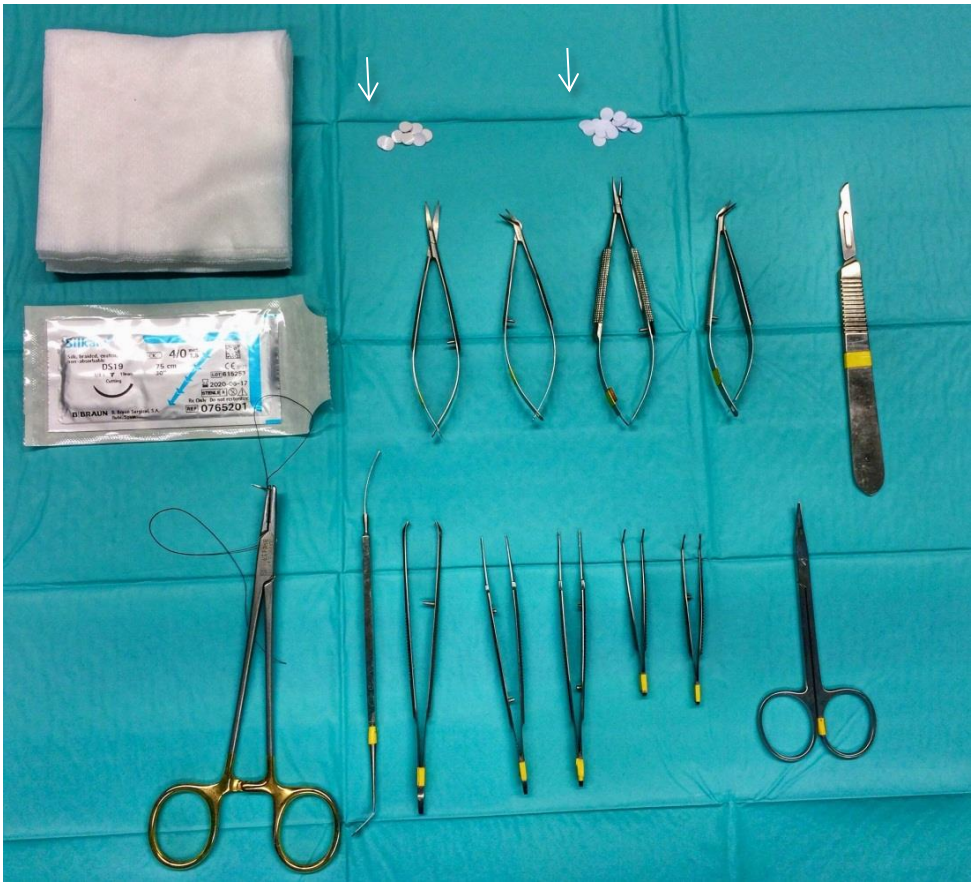

Figure 8 – Surgical instruments utilized in the implantation procedure (lint, scissor, scalpel, needle holder, tweezers, and suture thread) and the implant disks (white arrows).

2.2.3 HISTOLOGICAL ANALYSIS

The samples were fixed in 10% paraformaldehyde solution and prepared for the histological examination. In the histological examination, the fixed samples were embedded in paraffin and sectioned into a thickness of 5 μm and then stained with hematoxylin and eosin (HE) for general tissue structure and cell morphology, and Masson's trichrome (TM) staining to assess collagen deposition and capsule formation.

Histological analysis was conducted by one investigator, using a conventional diagnostic microscope (Nikon Eclipse E200). The tissue-biomaterial interface was evaluated by examination of the implantation bed and the peri-implant tissue.

A semi-quantitative methodology based on the total number of PMNs, macrophages, monocytes and lymphocytes, plasma cells and multinucleated giant cells per field was implemented to grade the intensity of the inflammatory response caused by both materials. The evaluator performing cell counts was blinded when examining the control and experimental

groups. The magnification of the light microscope was set at 400x. The inflammatory response was classified as follow [150], considering the mean of five evaluations by sample:

0 = minimally reactive (0 to 25 inflammatory cells per field),

1 = mildly reactive (25 to 50 inflammatory cells per field),

2 = moderately reactive (50 to 100 inflammatory cells per field),

3 = strongly reactive (inflammatory cells per field superior to 100).

Using digital images of the stained material, capsule thickness were determined by light microscopy using the image analysis software ZEN 2.3 lite (Carl Zeiss®, Oberkochen, Germany). Three random locations surround the implant were measured (µm) and the mean values were scored.

2.2.4 STATISTICAL ANALYSIS

For inflammatory reaction, semi-quantitative interpretations were presented descriptively. One-way ANOVA was used to determine the statistically significant differences of capsule thickness along the time in the experimental groups. Student's t-test with Tukey post hoc test was performed to compare the capsule thickness between day-7, day-14 and day-60 and the type of biomaterial. The results were considered significant when $p<0.05$.

2.3 RESULTS

Macroscopically, there were no signs of infection in any of the rats. However, six animals had samples exhibiting abscess formation close to the surgical incision when examined microscopically (Figure 9).

Some samples exhibited two different areas of inflammation, one corresponding to the surgical procedure and the other one surrounding the implant, mainly observed in the animals euthanized with 7 days. Usually, the inflammation response close to the implant was less intense than the response to the surgical procedure.

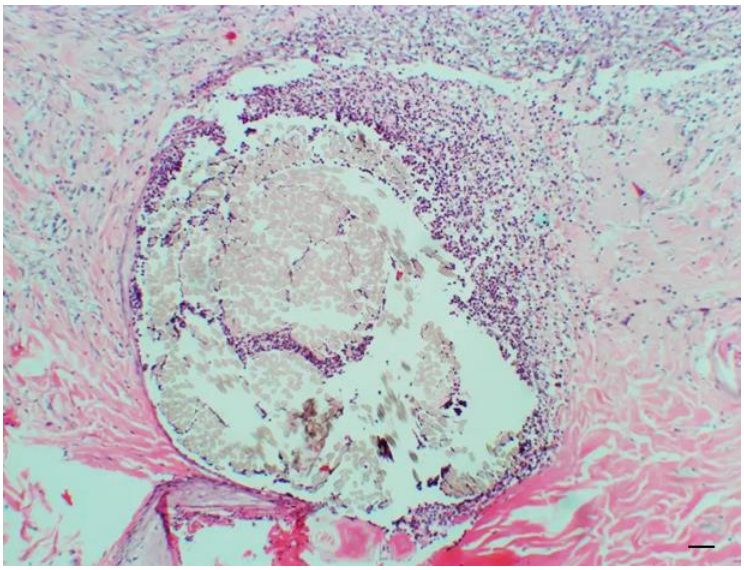

Figure 9 – Abscess formation close to the surgical incision – 7 days PLLA standard surface (scale bar 50µm).

A minimal to moderate inflammatory response was observed by HE staining for PLLA superhydrophobic surface. PLLA standard showed mild to moderate inflammatory response. At the day-7, the inflammatory reaction was classified as moderate reactive for both biomaterials (Figure 10); on the other hand, at the day-14 and -60, there were only scant inflammatory cells surrounding the implant surfaces (Figure 11). A reduction of the inflammatory process was verified after 60 days in comparison to 7 days for both groups, but this behavior was more important for the PLLA superhydrophobic group (Table 6).

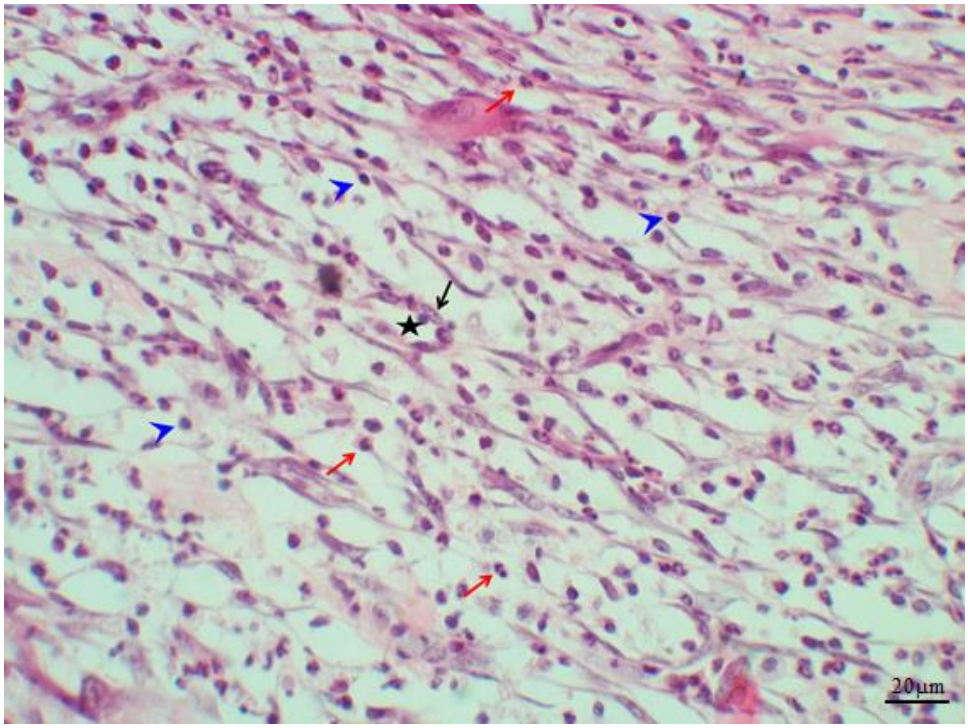

Figure 10 – Representative histological section (HE staining) of tissue reaction to the PLLA standard surface at day-7. Mononuclear cells (blue arrowheads), PMNs (red arrows), vessels (black star), endothelial cell (black arrow).

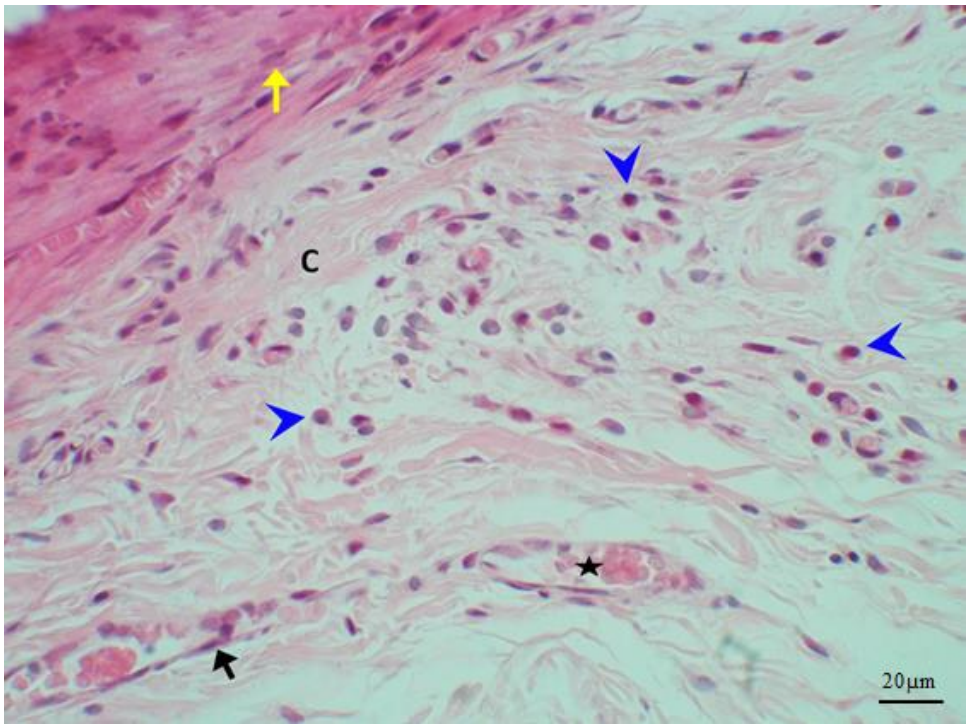

Figure 11 – Representative histological section (HE staining) of tissue reaction to the PLLA standard surface at day-60 close to the implant site. Mononuclear cells (blue arrowheads), fibroblast (yellow arrows), vessels (black star), endothelial cell (black arrow), collagen (C).

Table 6 – Inflammation score based on the number of inflammatory cells for PLLA standard and PLLA superhydrophobic in the day-7, day-14 and day-60.

Material	Day 7	Day 14	Day 60
PLLA Standard	Moderately reactive	Mildly reactive	Mildly reactive
PLLA Superhydrophobic	Moderately reactive	Mildly reactive	Minimally reactive

The TM staining showed the formation of a fibrous capsule surrounding both the PLLA standard and superhydrophobic surfaces at all the intervals. The mean of capsule thickness surrounding the PLLA implants was 42,2 ± 19,9 µm (SEM). The fibrous capsule at the day point 7 showed not well-organized, with a minimal and loose arrangement of collagen and many inflammatory cells between fibroblasts. At day-14, the capsule maintained similar features compared to day-7. Histologic analysis of day-60 post-surgery capsule demonstrated an implantation site surrounded by a well-organized fibrous capsule containing densely packed collagen fibers, several fibroblasts and few inflammatory cells (Figure 12).

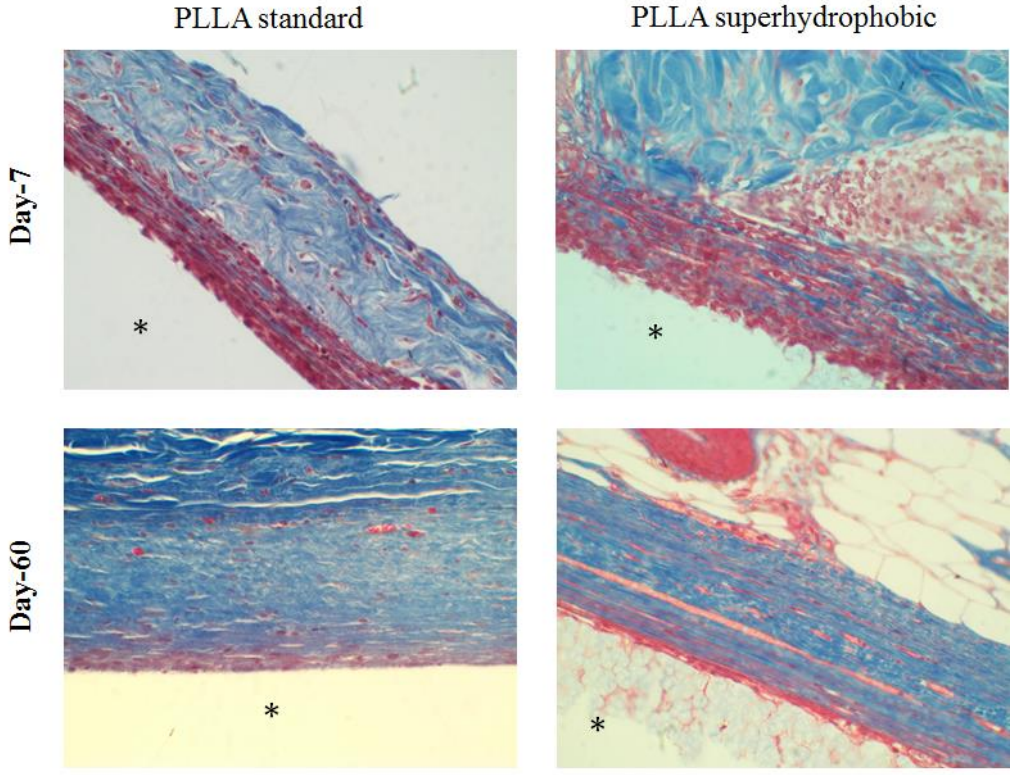

Figure 12 – TM staining of fibrous capsule at day-7 and day-60 of PLLA standard and superhydrophobic group. (*: implant site; 100x).

No statistical difference was demonstrated by Student's t-test and Tukey's test between the capsule thickness at day-7 and day-14, and between day-14 and day-60; however there was significant difference between day-7 and day-60. In these tests, the PLLA standard and PLLA superhydrophobic were considered together (Figure 13).

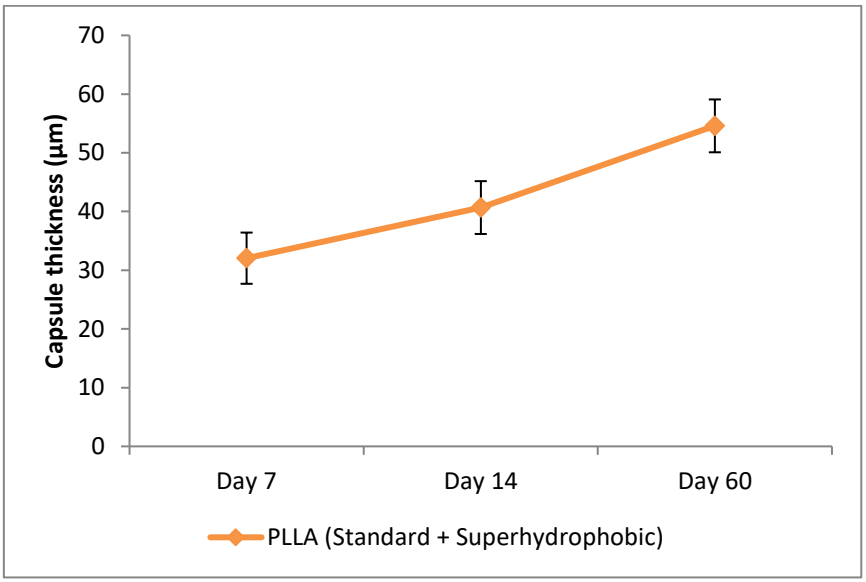

Figure 13 – Capsule thickness (µm) along the time considering the measurement of PLLA standard and superhydrophobic. Mean ± SEM.

The capsule thickness measurement analyzed by ANOVA revealed statistically difference along the time in the PLLA standard group ($p<0.001$), but not in the PLLA superhydrophobic group (Figure 14).

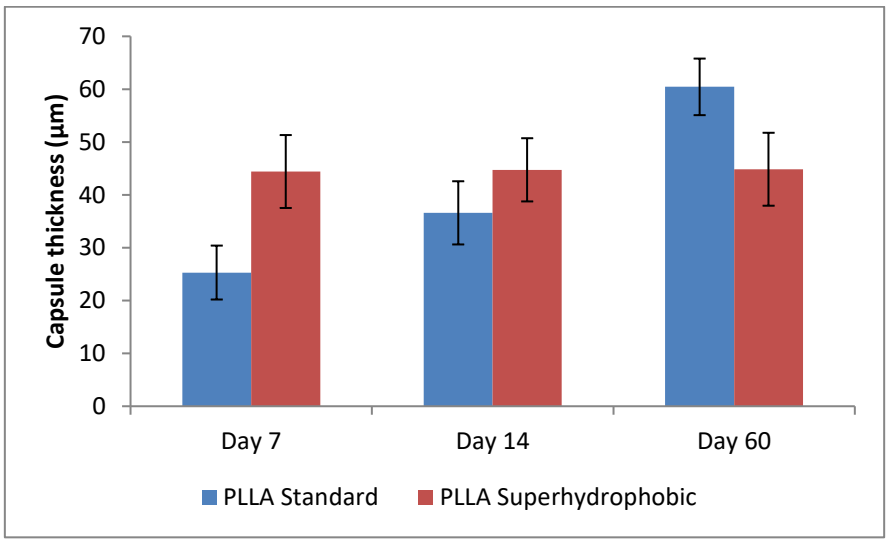

Figure 14 – Fibrous capsule thickness around PLLA standard and superhydrophobic 7, 14 and 60 days after implantation. Mean ± SEM.

The Tukey's test showed no statistical difference between day-7 and day-14 and between day-14 and day-60; however, significantly difference was found between day-7 and day-60 in the PLLA standard group. In summary, at day-60 capsules were thicker, with more densely arranged collagenous tissue in the PLLA standard group, compared with those at day-7. Moreover, it was not found a significant difference in the capsule thickness when comparing PLLA standard and PLLA superhydrophobic (Table 7).

Table 7 – Measurement of fibrous capsule thickness (µm) surround PLLA standard and superhydrophobic at day-7, day-14 and day-60. Data not connected by same letter are significantly different ($p<0.05$). Data are expressed as mean ± SEM.

	Capsule Thickness (µm)	
	PLLA Standard	**PLLA Superhydrophobic**
Day 7	25,3 ± 5,1 [b]	44,4 ± 6,9 [a,b]
Day 14	36,6 ± 5,9 [a,b]	44,7 ± 5,9 [a,b]
Day 60	60,4 ± 5,3 [a]	44,8 ± 6,9 [a,b]

No statistical significance was found by Student's t-test for the capsule thickness related with the type of biomaterial implanted when considering all the time points together (Figure 15).

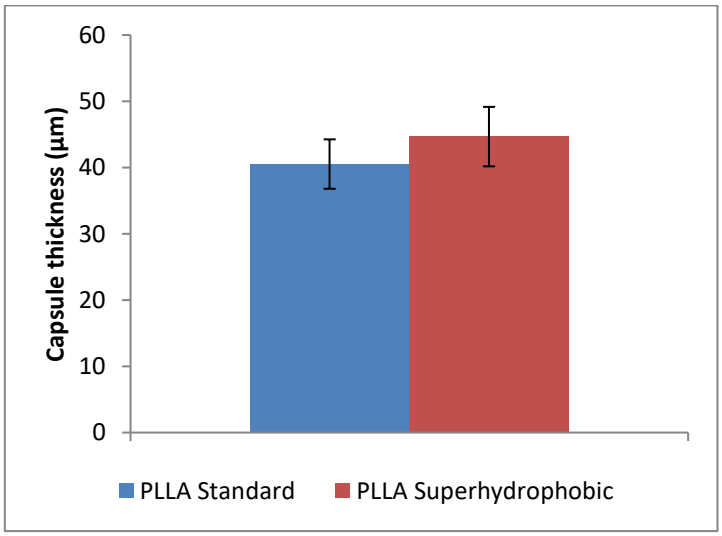

Figure 15 – Comparison between PLLA standard and superhydrophobic capsule thickness (µm). Mean +/- SEM.

The implant localization (subcutaneous at the level of scapula and pelvis) did not show influence in the capsule thickness and inflammation reaction.

2.4 DISCUSSION

Since many years, studies have been trying to associate the material surface properties to the inflammatory responses and features of capsule around the implants. However, the disparity between one study and another makes difficult to establish a reliable relation that fully clarify this question about so many different biomaterials with distinct features.

In this study, two different surfaces, PLLA hydrophobic and superhydrophobic, were implanted subcutaneously in rats and analyzed after 7, 14 and 60 days. The characteristics of the surfaces were not accessed by scanning electron microscopy, thus, we assumed the PLLA standard as smooth with water CA 71.3º ± 2.3º and superhydrophobic as rough with water CA 153.6º ± 1.9º, as previous described in [149], by the group that provided the material to perform this experimental research.

The numerous inflammatory cells in the first 7 days close to the implant site was compatible with a reaction due to the tissue disorganization caused by the surgical procedure, which was the reason of early inflammatory response after implantation. The score result of one of the samples exhibiting abscess formation close to the surgical incision may have been influenced by the infection, demonstrating an inflammation reaction higher than others. It was the only sample classified as strongly reactive with high number of PMNs. Abscesses are a frequent manifestation of *Staphylococcus aureus* skin and soft tissue infections and the neutrophils are the primary cellular host defense against it [151]. The other samples that demonstrated abscess formation did not appear to have been influenced by it, showing the same scores for other implantations sites with no signs of abscess.

At the day-7, the inflammatory reaction was classified as moderate reactive for both biomaterials. From the day 14, a reduction of the inflammatory process was verified being in agreement with the wound-healing process. The total number of infiltrating cells normally decreases as the inflammatory response resolves and progress to the fibrous encapsulation of the implanted material [152].

Biomaterial surface chemistry has been shown to modulate the amount of adsorbed proteins responsible for inflammation and foreign body reaction [153, 154]. Hydrophilicity is another feature that may influence serum proteins to adhere to materials and consequently modify the biological response, such as cell adhesion, proliferation, and function [155], influencing the biocompatibility. In this study, comparing hydrophobic and superhydrophobic surfaces, during the first 14 days, no difference was noticed between both biomaterials in the

inflammatory response. However, at the day-60, less inflammatory cells were observed in the superhydrophobic samples compared to hydrophobic ones.

Protein adsorption on superhydrophobic surfaces tended to be reduced when compared to more wettable surfaces [60] and this ability to resist protein adhesion demonstrated to decrease the number inflammatory cells adhering to biomaterials [156]. The results obtained by Roach et al. (2006) allowed observing lower adsorption of albumin and fibrinogen on superhydrophobic compared to hydrophobic surface [77]. The higher inflammatory reaction in the PLLA standard surface after 60 days could be explained by the tendency of protein adsorption on surfaces with moderate wettability signalizing the cells to attach and proliferate. Song et al. (2009) also demonstrated that superhydrophobic surfaces prepared by phase separation methods showed low cell adhesion [149], however, the results in this study did not show any difference in the early inflammatory response, indicating that cells can adhere on both surfaces, but they are tended to proliferate less in the superhydrophobic ones.

The topography of biomaterials was also reported influencing the behavior or proteins and cells attachment. In the study performed by Lourenço et al. (2012) the amount of protein adsorbed onto rough surfaces was significantly lower when compared with smooth surfaces of the same material [60]. As previously described, it is believed that these interactions between protein and biomaterial expose receptors for inflammatory cells, which then initiate the foreign body reactions [154].

Oliveira et al. (2011) demonstrated cell proliferation *in vitro* higher in hydrophilic surfaces, independently of being rough or smooth. The results highlighted the influence of wettability as the main responsible factor to explain the different cell behavior on smooth and rough surfaces [95]. Rosa, Beloti & van Noort (2003), studying the influence of hydroxyapatite (HA) topography on osteoblastic differentiation observed surfaces with a more regular topography favoring cell proliferation. However, different degrees of microporosity show to modify the behavior of cells [157]. Ranella et al. (2010) observed that fibroblast spreading becomes optimum on low-rough substrates, independently of their wettability and chemistry. Additionally, a modification in surface energy could switch the cell behavior in materials with the same degree of roughness [158]. Considering the PLLA superhydrophobic as a rough surface, the lowest inflammation score after 60 days post-implantation could be justified by its wettability and roughness. However, some studies showed contradictory results when analyzing other surfaces with extreme wettabilities and different cells and proteins. According to Wang et al. (2014), the smooth surfaces might produce less stimulation to macrophages than the rough

surface [155]. Recum et al. (1996) demonstrated that fibroblasts seem to like to interdigitate and anchor to textured surfaces, especially in certain size ranges [159]. An interesting review made by Song and Mano (2013) gives an overview of recent studies in this sense [160], showing contradictories results depending of the cell type, the material used, chosen protein, etc.

Despite the indication that material surface may determine how proteins and cells interact with biomaterials the influence of wettability still remains unclear. Kim et al. (2007) assessed the host tissue response to PLGA in rats and demonstrated severe post-implantation inflammation in the surfaces with higher water CA compared to hydrophilic ones. They assumed the degradation products of PLGA as the cause for the undesirable inflammatory response [161]. In fact, the degradation products of PLLA reduce local pH and induce inflammatory reaction [57]. However, superhydrophobic surfaces provide a weak interaction between body fluid and the implanted biomaterial, making the degradation rate slower [63]. Once morphologic changes are observed after 4 weeks indicating degradation process [162], this could also explain the lower inflammation score at day-60 in the PLLA superhydrophobic group compared to the PLLA standard. On the other hand, Bos et al. (1991) indicated the resorption of PLLA starting close to 26 weeks and it did not give rise to any histologically detectable reaction up to 104 weeks, illustrating the good biocompatibility of PLLA and its degradation products in rats [163]. In this sense, a long-term study should be performed comparing PLLA standard and superhydrophobic in order to confirm this hypothesis between these two materials.

The normal foreign body reaction consists of fibroblastic proliferation and collagen deposition subjacent to the surface implanted [138]. This study had shown the formation of a fibrous capsule surrounding both the PLLA standard and superhydrophobic surfaces at all the intervals, but with differences in cells and collagen arrangement between the time point 7 and 60, compatible with a normal wound healing. In the early inflammation (5 to 10 days post implantation), fibroblasts access the wound site to switch the provisional matrix with granulation tissue composed of fibronectin and collagen. While neovascularization appears, fibroblasts differentiate into myofibroblasts and contract the matrix to repair the tissue and approximate the wound margins. The rich collagen left from cells' apoptosis forms the scar tissue that is slowly remodeled in the following months [164].

The capsule thickness measurement demonstrated significant difference along the time in the PLLA standard group, but not in the PLLA superhydrophobic group. Andersson et al. (2008) suggested that a thick layer of fibrous tissue is an effect of the protein-surface interaction

seen in the early stages of inflammation and the amount of cells associated to the surface could be directly related to the capsule thickness. They compared different materials with different contact angles and the results showed more cells associated to the material less hydrophobic, with consequently thicker capsule [165]. In our study, no difference in the inflammatory response was found in the early stages and the posterior capsule thickness between both groups; however the degradation products of PLLA standard released to the surrounding tissue could explain the increase of capsule thickness in this group comparing the day-7 and day-60, once these products keep stimulating the inflammation and the degradation rate of superhydrophobic surfaces is theoretically lower. Grayson et al. (2004) studied the degradation rates of PLA, classified as slowly degrading, and PGA, classified as rapidly degrading, and the results showed that fibrous capsule thickness of the PGA increased in the first days, decreasing after resolution of inflammatory response, while PLA fibrous capsule thickness steadily increased [166]. Moreover, the difficulty in establishing the capsule borders in the early periods of inflammation because of cell disarray could influence the measurement of it. Others studies performed analysis of fibroblasts and blood vessels influencing the fibrous tissue thickness [167], however, these analyzes were not performed in this study, as proteins as well.

According to Suska et al. (2008) the movement of implants in the subcutaneous compartment can also have an effect on capsule thickness [167]. The movement existed initially for both materials must be similar; therefore, the significantly thicker capsule around PLLA superhydrophobic might moderate mechanical shear influencing negatively further development. On the other hand, the thinner capsule surrounding PLLA standard could explain the increase in the thickness from day-7 to day-60, since the movement could stimulate inflammatory process.

Further, there was no significant difference related with the type of biomaterial implanted, when analyzing all measurements of the thickness capsule. The *in vivo* response evaluated from inflammatory infiltrates and capsule thickness revealed that PLLA standard and PLLA superhydrophobic did not cause severe inflammation and demonstrated good biocompatibility for biomedical applications.

A potential limitation of this study is the relatively small numbers of rats used for each time point (n=3). A larger sample size allows a more precise estimate of the treatment effect and make easier to assess the representativeness of the sample and to generalize the results [168]. Moreover, the difficulty in cutting the samples with a microtome, because of the hardness of PLLA, damaged some soft tissues and fragmented some implants making impossible the

employment of the samples in this study. It was previously described that specimens containing synthetic biomaterials with distinct hardness make the preparation of adequate microscope slides for histopathology challenging [169] and conventional techniques applied for this purpose, such as sectioning using a microtome, are the main raison for the difficulty to obtain scientific data, particularly during an *in vivo* study [170]. Chai et al. (2011) highlighted some techniques to remove the biomaterial before sectioning the samples with the objective to overcome this difficulty (e.g. fracture technique, mechanical separation, cryofracturing technique and electrochemical dissolution/electropolishing) [171]. PLLA was already studied before in the sense of access the inflammatory response *in vivo*, however the methods do not describe a specific technique to prepare the samples for histology [61, 155, 172].

The analysis about materials properties influencing the cells and proteins behavior is a complex task due to the difficulty in isolating the influence of each parameter and in comparing the studies with so many variables. Evaluation of the biocompatibility of the same biomaterial with different characteristics is important way to understand how the organism reacts against some properties changes to improve the biomaterials in the future.

2.5 CONCLUSION

In this study, we have been focused in demonstrate the inflammatory response in rats implanting PLLA films with different wettability in the subcutaneous tissue.

Histological finds provided evidences of a good acceptability for both materials, hydrophobic and superhydrophobic, because no signs of severe inflammation were found and a relative long-term treatment was well tolerated. In conclusion, there was a moderately inflammatory response to the implanted material at initial periods which decreased to mild/minimal at the final period considered 2 months after implantation. There was a well-ordered host response with wound healing signs along the time.

However, future studies applied to a greater number of animals, may be needed to confirm the results. Apply a technique to remove the implant could benefit the samples' quality. Moreover, protein adsorption and cell type adhesion investigation would give more information about the relation between these biodegradable materials and inflammatory response and material characteristics analysis would improve the discussion and the comparison with other studies.

REFERENCES

[1] Langer, R., & Vacanti, J. P. (1993). Tissue engineering. *Science*, 920-926.

[2] Daar, A. S., & Greenwood, H. L. (2007). A proposed definition of regenerative medicine. *Journal of Tissue Engineering and Regenerative Medicine, 1*(3), 179-184.

[3] Salgado, A. J., Oliveira, J. M., Martins, A., Teixeira, F. G., Silva, N. A., Neves, N. M., et al. (2013). Tissue Engineering and Regenerative Medicine: past, present, and future. In S. Geuna, I. Perroteau, P. Tos, & B. Battiston, *Tissue Engineering of the Peripheral Nerve: Stem Cells and Regeneration Promoting Factors* (Vol. 108, pp. 1-33). Elsevier.

[4] Neves, L. S., Rodrigues, M. T., Reis, R. L., & Gomes, M. E. (2016). Current approaches and future perspectives on strategies for the development of personalized tissue engineering therapies. *Expert Review of Precision Medicine and Drug Development*, 1(1), 93-108.

[5] Correia, S. I., Pereira, H., Silva-Correia, J., Van Dijk, C. N., Espregueira-Mendes, J., Oliveira, J. M., et al. (2014). Current concepts: tissue engineering and regenerative medicine applications in the ankle joint. *Journal of Royal Society Interface, 11*.

[6] Plunkett, N., & O'Brien, F. J. (2011). Bioreactors in tissue engineering. *Technology and Health Care, 19*, 55-69.

[7] Şenel, S., & McClure, S. J. (2004). Potencial applications of chitosan in veterinary medicine. *Advanced Drug Delivery Reviews, 56*, 1467-1480.

[8] Torricelli, P., Fini, M., Filardo, G., Tschon, M., Pischedda, M., Pacorini, A., et al. (2011). Regenerative medicine for the treatment of musculoskeletal. *International Orthopaedics, 35*, 1569-1576.

[9] Badylak, S. F. (2004). Extracellular Matrix As a Scaffold for Tissue Engineering in Veterinary Medicine: Applications to Soft Tissue Healing. *Clinical Techniques in Equine Practice, 3*(2), 173-181.

[10] O'Brien, F. J. (2011). Biomaterials & scaffolds for tissue engineering. *Materials Today, 14*(3), 88-95.

[11] Katari, R. S., Peloso, A., & Orlando, G. (2014). Tissue Engineering. *Advances in Surgery, 48*, 137-154.

[12] El-Kassaby, A. W., Retik, A. B., Yoo, J. J., & Atala, A. (2003). Urethral stricture repair with an off-the-self collagen matrix. *The Journal of Urology, 169*, 170-173.

[13] Niknejad, H., Peirovi, H., Jorjani, M., Ahmadiani, A., Ghanavi, J., & Seifalian, A. M. (2008). Properties of the amniotic membrane for potential use in tissue engineering. *Eur Cells Mater*, 15, 88-99.

[14] Marois Y, Guidoin R. Biocompatibility of Polyurethanes. In: *Madame Curie Bioscience Database* [Internet]. Austin (TX): Landes Bioscience; 2000-2013. Retrieved October 12, 2017. Available from: https://www.ncbi.nlm.nih.gov/books/NBK6422/

[15] Lee, M. H., Arcidiacono, J. A., Bilek, A. M., Wille, J. J., Hamill, C. A., Wonnacott, K. M., et al. (2010). Considerations for tissue-engineered and regenerative medicine product development prior to clinical trials in the United States. *Tissue Engineering Part B: Reviews.*, *16*(1), 41-54.

[16] Pertici, G. (2016). Introduction to bioresorbable polymers for biomedical applications. In G. Perale, & J. Hilborn, *Bioresorbable Polymers for Biomedical Applications.* Woodhead Publishing.

[17] Vert, M. (2005). Aliphatic polyesters: great degradable polymers that cannot do everything. *Biomacromolecules*, 6(2), 538-546.

[18] Navarro, M., Michiardi, A., Castano, O., & Planell, J. A. (2008). Biomaterials in orthopaedics. *Journal of the Royal Society Interface*, 5(27), 1137-1158.

[19] Bat, E., Zhang, Z., Feijen, J., Grijpma, D. W., & Poot, A. A. (2014). Biodegradable elastomers for biomedical applications and regenerative medicine. *Regenerative Medicine*, 9(3), 385-398.

[20] Lasprilla, A. J., Martinez, G. A., Lunelli, B. H., Jardini, A. L., & Maciel Filho, R. (2012). Poly-lactic acid synthesis for application in biomedical devices—A review. *Biotechnology advances*, *30*(1), 321-328.

[21] Roether, J. A., Boccaccini, A. R., Hench, L. L., Maquet, V., Gautier, S., & Jérôme, R. (2002). Development and in vitro characterisation of novel bioresorbable and bioactive composite materials based on polylactide foams and Bioglass® for tissue engineering applications. *Biomaterials*, *23*(18), 3871-3878.

[22] Zhou, Z. H., Ruan, J. M., Zhou, Z. C., & Shen, X. J. (2007). Bioactivity of bioresorbable composite based on bioactive glass and poly-L-lactide. *Transactions of Nonferrous Metals Society of China*, *17*(2), 394-399.

[23] Lopes, M. S., Jardini, A. L., & Maciel Filho, R. (2012). Poly (lactic acid) production for tissue engineering applications. *Procedia Engineering*, *42*, 1402-1413.

[24] Ulery, B. D., Nair, L. S., & Laurencin, C. T. (2011). Biomedical applications of biodegradable polymers. *Journal of polymer science Part B: polymer physics*, *49*(12), 832-864.

[25] Nakamura, T., Hitomi, S., Watanabe, S., Shimizu, Y., Jamshidi, K., Hyon, S. H., & Ikada, Y. (1989). Bioabsorption of polylactides with different molecular properties. *Journal of Biomedical Materials Research Part A*, *23*(10), 1115-1130.

[26] Willbold, E., Kalla, K., Bartsch, I., Bobe, K., Brauneis, M., Remennik, S., et al. (2013). Biocompatibility of rapidly solidified magnesium alloy RS66 as a temporary biodegradable metal. *Acta Biomaterialia*, *9*(10), 8509-8517.

[27] Artzi, N., Oliva, N., Puron, C., Shitreet, S., Artzi, S., bon Ramos, A., et al. (2011). In vivo and in vitro tracking of erosion in biodegradable materials using non-invasive fluorescence imaging. *Nature Materials*, *10*(9), 704.

[28] Latour, R. A. (2005). Biomaterials: protein-surface interactions. *Encyclopedia of biomaterials and biomedical engineering*, *1*, 270-278.

[29] Mano, J. F., Silva, G. A., Azevedo, H. S., Malafaya, P. B., Sousa, R. A., Silva, S. S., et al. (2007). Natural origin biodegradable systems in tissue engineering and regenerative medicine: present status and some moving trends. *Journal of Royal Society Interface*, *4*(17), 999-1030.

[30] Gilbert, T. W., Sellaro, T. L., & Badylak, S. F. (2006). Decellularization of tissues and organs. *Biomaterials*, *27*(19), 3675-3683.

[31] Ha, T. L. B., Quan, T. M., & Vu, D. N. (2013). Naturally derived biomaterials: Preparation and application. In *Regenerative medicine and tissue engineering*. Intech.

[32] Visser, S. A., Hergenrother, R. W., & Cooper, S. L. (1996). Polymers. In B. D. Ratner, A. S. Hoffman, F. J. Schoen, & J. E. Lemons, *Biomaterials Science: An Introduction to Materials in Medicine* (p. 50). Academic Press.

[33] Angelova, N., & Hunkeler, D. (1999). Rationalizing the design of polymeric biomaterials. *Trends in biotechnology*, *17*(10), 409-421.

[34] Costa-Pinto, A. R., Reis, R. L., & Neves, N. M. (2011). Scaffolds based bone tissue engineering: the role of chitosan. *Tissue Engineering Part B: Reviews*, *17*(5), 331-347.

[35] Lazzeri, L., Barbani, N., Cascone, M. G., Lupinacci, D., Giusti, P., & Laus, M. (1994). Physico-chemical and mechanical characterization of hydrogels of poly (vinyl alcohol) and hyaluronic acid. *Journal of Materials Science: Materials in Medicine*, *5*(12), 862-867.

[36] Ikada, Y., & Tsuji, H. (2000). Biodegradable polyesters for medical and ecological applications. *Macromolecular rapid communications, 21*(3), 117-132.

[37] Wolinsky, J. B., Colson, Y. L., & Grinstaff, M. W. (2012). Local drug delivery strategies for cancer treatment: gels, nanoparticles, polymeric films, rods, and wafers. *Journal of controlled release, 159*(1), 14-26.

[38] Greenstein, G., & Polson, A. (1998). The role of local drug delivery in the management of periodontal diseases: a comprehensive review. *Journal of periodontology, 69*(5), 507-520.

[39] Hubbell, J. A. (1998). Synthetic biodegradable polymers for tissue engineering and drug delivery. *Current Opinion in Solid State and Materials Science, 3*(3), 246-251.

[40] Pillai, O., & Panchagnula, R. (2001). Polymers in drug delivery. *Current opinion in chemical biology, 5*(4), 447-451.

[41] Yannas, J. V. (1996). Natural Materials. In B. D. Ratner, A. S. Hoffman, F. J. Schoen, & J. E. Lemons, *Biomaterials Science: An Introduction to Materials in Medicine* (p. 84). Academic Press.

[42] Dang, J. M., & Leong, K. W. (2006). Natural polymers for gene delivery and tissue engineering. *Advanced drug delivery reviews, 58*(4), 487-499.

[43] Li, M., Mitra, D., Kang, E. T., Lau, T., Chiong, E., & Neoh, K. G. (2017). Thiol-ol Chemistry for Grafting of Natural Polymers to Form Highly Stable and Efficacious Antibacterial Coatings. *ACS applied materials & interfaces, 9*(2), 1847-1857.

[44] Sabir, M. I., Xu, X., & Li, L. (2009). A review on biodegradable polymeric materials for bone tissue engineering applications. *Journal of materials science, 44*(21), 5713-5724.

[45] Widner, B., Behr, R., Von Dollen, S., Tang, M., Heu, T., Sloma, A., et al. (2005). Hyaluronic acid production in Bacillus subtilis. *Applied and Environmental Microbiology, 71*(7), 3747-3752.

[46] Kobayashi, S. (2007). New developments of polysaccharide synthesis via enzymatic polymerization. *Proceedings of the Japan Academy, Series B, 83*(8), 215-247.

[47] Hacker, M., & Mikos, A. G. (2011). Synthetic Polymers. In A. Atala, R. Lanza, J. A. Thomson, & R. M. Nerem, *Principles of Regenerative Medicine* (2nd ed., pp. 587-622). Academic Press.

[48] Sionkowska, A. (2011). Current research on the blends of natural and synthetic polymers as new biomaterials. *Progress in Polymer Science, 36*(9), 1254-1276.

[49] Gunatillake, P. A., & Adhikari, R. (2003). Biodegradable synthetic polymers for tissue engineering. *Eur Cell Mater*, *5*(1), 1-16.

[50] Park, J. H., Jung, J. W., Kang, H. W., & Cho, D. W. (2014). Indirect three-dimensional printing of synthetic polymer scaffold based on thermal molding process. *Biofabrication*, *6*(2), 025003.

[51] Tian, H., Tang, Z., Zhuang, X., Chen, X., & Jing, X. (2012). Biodegradable synthetic polymers: preparation, functionalization and biomedical application. *Progress in Polymer Science*, *37*(2), 237-280.

[52] Griffith, L. G. (2000). Polymeric biomaterials. *Acta materialia*, *48*(1), 263-277.

[53] Kohn, J., & Langer, R. (1996). Bioresorbable and Bioerodible Materials. In B. D. Ratner, A. S. Hoffman, F. J. Schoen, & J. E. Lemons, *Biomaterials Science: An Introduction to Materials in Medicine* (p. 69). Academic Press.

[54] Lee, S. J., & Atala, A. (2015). Where Are We Going? Future Trends and Challenges. *Polymers in Regenerative Medicine: Biomedical Applications from Nano-to Macro-Structures*, 367-389.

[55] Liu, H., Slamovich, E. B., & Webster, T. J. (2006). Less harmful acidic degradation of poly (lactic-co-glycolic acid) bone tissue engineering scaffolds through titania nanoparticle addition. *International journal of nanomedicine*, *1*(4), 541.

[56] Hamad, K., Kaseem, M., Yang, H. W., Deri, F., & Ko, Y. G. (2015). Properties and medical applications of polylactic acid: A review. *Express Polymer Letters*, *9*(5), 435–455.

[57] Finniss, A., Agarwal, S., & Gupta, R. (2016). Retarding hydrolytic degradation of polylactic acid: Effect of induced crystallinity and graphene addition. *Journal of Applied Polymer Science*, *133*(43).

[58] Rasal, R. M., Janorkar, A. V., & Hirt, D. E. (2010). Poly (lactic acid) modifications. *Progress in polymer science*, *35*(3), 338-356.

[59] Vieira, A. C., Vieira, J. C., Guedes, R. M., & Marques, A. T. (2010). Degradation and viscoelastic properties of PLA-PCL, PGA-PCL, PDO and PGA fibres. In *Materials Science Forum* (Vol. 636, pp. 825-832). Trans Tech Publications.

[60] Lourenço, B. N., Marchioli, G., Song, W., Reis, R. L., van Blitterswijk, C. A., Karperien, M., van Apeldoorn, A. & Mano, J. F. (2012). Wettability influences cell behavior on superhydrophobic surfaces with different topographies. *Biointerphases*, *7*(1-4), 1-11.

[61] Ishii, D., Ying, T. H., Mahara, A., Murakami, S., Yamaoka, T., Lee, W. K., & Iwata, T. (2008). In vivo tissue response and degradation behavior of PLLA and stereocomplexed PLA nanofibers. *Biomacromolecules*, *10*(2), 237-242.

[62] Leroy, A., Ribeiro, S., Grossiord, C., Alves, A., Vestberg, R. H., Salles, V., et al. (2017). FTIR microscopy contribution for comprehension of degradation mechanisms in PLA-based implantable medical devices. *Journal of Materials Science: Materials in Medicine, 28*(6), 87.

[63] Neumann, I. A., Flores-Sahagun, T. H. S., & Ribeiro, A. M. (2017). Biodegradable poly (l-lactic acid)(PLLA) and PLLA-3-arm blend membranes: The use of PLLA-3-arm as a plasticizer. *Polymer Testing*, *60*, 84-93.

[64] Andersson, S. R., Hakkarainen, M., Inkinen, S., Södergård, A., & Albertsson, A. C. (2010). Polylactide stereocomplexation leads to higher hydrolytic stability but more acidic hydrolysis product pattern. *Biomacromolecules*, *11*(4), 1067-1073.

[65] Cam, D., Hyon, S. H., & Ikada, Y. (1995). Degradation of high molecular weight poly (L-lactide) in alkaline medium. *Biomaterials*, *16*(11), 833-843.

[66] Davachi, S. M., Kaffashi, B., Torabinejad, B., & Zamanian, A. (2016). In-vitro investigation and hydrolytic degradation of antibacterial nanocomposites based on PLLA/triclosan/nano-hydroxyapatite. *Polymer*, *83*, 101-110.

[67] Lembeck, B., & Wülker, N. (2005). Severe cartilage damage by broken poly–L–lactic acid (PLLA) interference screw after ACL reconstruction. *Knee Surgery, Sports Traumatology, Arthroscopy*, *13*(4), 283-286.

[68] Bergsma, J. E., De Bruijn, W. C., Rozema, F. R., Bos, R. R. M., & Boering, G. (1995). Late degradation tissue response to poly (L-lactide) bone plates and screws. *Biomaterials*, *16*(1), 25-31.

[69] Funt, D., & Pavicic, T. (2013). Dermal fillers in aesthetics: an overview of adverse events and treatment approaches. *Clinical, cosmetic and investigational dermatology*, *6*, 295.

[70] Haneke, E. (2015). Managing complications of fillers: rare and not-so-rare. *Journal of cutaneous and aesthetic surgery*, *8*(4), 198.

[71] Giusti, P., Lazzeri, L., Barbani, N., Lelli, L., De Petris, S., & Cascone, M. G. (1994, February). Blends of natural and synthetic polymers: a new route to novel biomaterials. In *Macromolecular Symposia* (Vol. 78, No. 1, pp. 285-297). Hüthig & Wepf Verlag.

[72] Giusti, P., Lazzeri, L., Cascone, M. G., Barbani, N., & Cristallini, C. (1995). Bioartificial Polymeric Materials: Natural and Synthetic World Joining in Biomaterials Research. In *Polymers and Other Advanced Materials* (pp. 563-569). Springer US.

[73] Zhang, X., Shi, F., Niu, J., Jiang, Y., & Wang, Z. (2008). Superhydrophobic surfaces: from structural control to functional application. *Journal of Materials Chemistry*, *18*(6), 621-633.

[74] Marmur, A. (2012). Hydro-hygro-oleo-omni-phobic? Terminology of wettability classification. *Soft Matter*, *8*(26), 6867-6870.

[75] Rupp, F., Gittens, R. A., Scheideler, L., Marmur, A., Boyan, B. D., Schwartz, Z., & Geis-Gerstorfer, J. (2014). A review on the wettability of dental implant surfaces I: theoretical and experimental aspects. *Acta Biomaterialia*, *10*(7), 2894-2906.

[76] Liu, H., Gao, S.-W., Cai, J.-S., He, C.-L., Mao, J.-J., Zhu, T.-X., et al. (2016). Recent progress in fabrication and applications of syperhydrophobic coating on cellulose-based substrates. *Materials*, *9*(3), 124.

[77] Roach, P., Shirtcliffe, N. J., Farrar, D., & Perry, C. C. (2006). Quantification of surface-bound proteins by fluorometric assay: comparison with quartz crystal microbalance and amido black assay. *The Journal of Physical Chemistry B*, *110*(41), 20572-20579.

[78] K Webb, H., Hasan, J., K Truong, V., J Crawford, R., & P Ivanova, E. (2011). Nature inspired structured surfaces for biomedical applications. *Current Medicinal Chemistry*, *18*(22), 3367-3375.

[79] Barthlott, W., & Neinhuis, C. (1997). Purity of the sacred lotus, or escape from contamination in biological surfaces. *Planta*, *202*(1), 1-8.

[80] Feng, L., Zhang, Y., Xi, J., Zhu, Y., Wang, N., Xia, F., & Jiang, L. (2008). Petal effect: a superhydrophobic state with high adhesive force. *Langmuir*, *24*(8), 4114-4119.

[81] Ma, M., & Hill, R. M. (2006). Superhydrophobic surfaces. *Current opinion in colloid & interface science*, *11*(4), 193-202.

[82] Gasperini, L., Mano, J. F., & Reis, R. L. (2014). Natural polymers for the microencapsulation of cells. *Journal of the royal society Interface*, *11*(100), 20140817.

[83] Willner, I. (2017). Stimuli-controlled hydrogels and their applications. *Accounts of Chemical Research*, *50*, 657−658.

[84] Cheung, H. Y., Lau, K. T., Lu, T. P., & Hui, D. (2007). A critical review on polymer-based bio-engineered materials for scaffold development. *Composites Part B: Engineering*, *38*(3), 291-300.

[85] Ahearne, M. (2014). Introduction to cell–hydrogel mechanosensing. *Interface focus*, *4*(2), 20130038.

[86] Maitra, J., & Shukla, V. K. (2014). Cross-linking in hydrogels-a review. *American Journal of Polymer Science*, *4*(2), 25-31.

[87] Khakbaz, M., Hejazi, I., Seyfi, J., Jafari, S. H., Khonakdar, H. A., & Davachi, S. M. (2015). A novel method to control hydrolytic degradation of nanocomposite biocompatible materials via imparting superhydrophobicity. *Applied Surface Science*, *357*, 880-886.

[88] Sun, T., Tan, H., Han, D., Fu, Q., & Jiang, L. (2005). No platelet can adhere—largely improved blood compatibility on nanostructured superhydrophobic surfaces. *Small*, *1*(10), 959-963.

[89] Gittens, R. A., Scheideler, L., Rupp, F., Hyzy, S. L., Geis-Gerstorfer, J., Schwartz, Z., & Boyan, B. D. (2014). A review on the wettability of dental implant surfaces II: biological and clinical aspects. *Acta Biomaterialia*, *10*(7), 2907-2918.

[90] Gentleman, M. M., & Gentleman, E. (2014). The role of surface free energy in osteoblast–biomaterial interactions. *International Materials Reviews*, *59*(8), 417-429.

[91] Sartoretto, S. C., Alves, A. T. N. N., Resende, R. F. B., Calasans-maia, J., Granjeiro, J. M., & Calasans-maia, M. D. (2015). Early osseointegration driven by the surface chemistry and wettability of dental implants. *Journal of Applied Oral Science*, *23*(3), 279-287.

[92] Mano, J. F. (2008). Stimuli-responsive polymeric systems for biomedical applications. *Advanced Engineering Materials*, *10*(6), 515-527.

[93] Sawase, T., Jimbo, R., Baba, K., Shibata, Y., Ikeda, T., & Atsuta, M. (2008). Photo-induced hydrophilicity enhances initial cell behavior and early bone apposition. *Clinical oral implants research*, *19*(5), 491-496.

[94] Ishizaki, T., Saito, N., & Takai, O. (2010). Correlation of cell adhesive behaviors on superhydrophobic, superhydrophilic, and micropatterned superhydrophobic/superhydrophilic surfaces to their surface chemistry. *Langmuir*, *26*(11), 8147-8154.

[95] Oliveira, S. M., Song, W., Alves, N. M., & Mano, J. F. (2011). Chemical modification of bioinspired superhydrophobic polystyrene surfaces to control cell attachment/proliferation. *Soft Matter*, *7*(19), 8932-8941.

[96] Ji, Y. W., Hong, S. H., Chung, D. Y., Kim, E. K., & Lee, H. K. (2014). Comparison of surface roughness and bacterial adhesion between cosmetic contact lenses and conventional contact lenses. *Journal of the Korean Ophthalmological Society*, *55*(5), 646-655.

[97] Zhang, X., Wang, L., & Levänen, E. (2013). Superhydrophobic surfaces for the reduction of bacterial adhesion. *Rsc Advances*, *3*(30), 12003-12020.

[98] Aykent, F., Yondem, I., Ozyesil, A. G., Gunal, S. K., Avunduk, M. C., & Ozkan, S. (2010). Effect of different finishing techniques for restorative materials on surface roughness and bacterial adhesion. *The Journal of Prosthetic Dentistry*, *103*(4), 221-227.

[99] Scarano, A., Piattelli, M., Caputi, S., Favero, G. A., & Piattelli, A. (2004). Bacterial adhesion on commercially pure titanium and zirconium oxide disks: an in vivo human study. *Journal of Periodontology*, *75*(2), 292-296.

[100] Dantas, L. C. D. M., Silva-Neto, J. P. D., Dantas, T. S., Naves, L. Z., das Neves, F. D., & da Mota, A. S. (2016). Bacterial adhesion and surface roughness for different clinical techniques for acrylic polymethyl methacrylate. *International Journal of Dentistry*, *2016*.

[101] Bollenl, C. M., Lambrechts, P., & Quirynen, M. (1997). Comparison of surface roughness of oral hard materials to the threshold surface roughness for bacterial plaque retention: a review of the literature. *Dental Materials*, *13*(4), 258-269.

[102] Tang, P., Zhang, W., Wang, Y., Zhang, B., Wang, H., Lin, C., & Zhang, L. (2011). Effect of superhydrophobic surface of titanium on Staphylococcus aureus adhesion. *Journal of Nanomaterials*, *2011*, 2.

[103] Sousa, C., Rodrigues, D., Oliveira, R., Song, W., Mano, J. F., & Azeredo, J. (2011). Superhydrophobic poly (L-lactic acid) surface as potential bacterial colonization substrate. *AMB express*, *1*(1), 34.

[104] Wennerberg, A., & Albrektsson, T. (2009). Effects of titanium surface topography on bone integration: a systematic review. *Clinical oral implants research*, *20*(s4), 172-184.

[105] Falde, E. J., Yohe, S. T., Colson, Y. L., & Grinstaff, M. W. (2016). Superhydrophobic materials for biomedical applications. *Biomaterials*, *104*, 87-103.

[106] Huber, D. L., Manginell, R. P., Samara, M. A., Kim, B. I., & Bunker, B. C. (2003). Programmed adsorption and release of proteins in a microfluidic device. *Science*, *301*(5631), 352-354.

[107] Lima, A. C., Custódio, C. A., Alvarez-Lorenzo, C., & Mano, J. F. (2013). Biomimetic methodology to produce polymeric multilayered particles for biotechnological and biomedical applications. *Small*, *9*(15), 2487-2492.

[108] Song, W., Lima, A. C., & Mano, J. F. (2010). Bioinspired methodology to fabricate hydrogel spheres for multi-applications using superhydrophobic substrates. *Soft Matter*, *6*(23), 5868-5871.

[109] Neto, A. I., Custódio, C. A., Song, W., & Mano, J. F. (2011). High-throughput evaluation of interactions between biomaterials, proteins and cells using patterned superhydrophobic substrates. *Soft Matter*, *7*(9), 4147-4151.

[110] Oliveira, M. B., Neto, A. I., Correia, C. R., Rial-Hermida, M. I., Alvarez-Lorenzo, C., & Mano, J. F. (2014). Superhydrophobic chips for cell spheroids high-throughput generation and drug screening. *ACS Applied Materials & Interfaces*, *6*(12), 9488-9495.

[111] Li, L., Tian, J., Li, M., & Shen, W. (2013). Superhydrophobic surface supported bioassay–An application in blood typing. *Colloids and Surfaces B: Biointerfaces*, *106*, 176-180.

[112] Nair, L. S., & Laurencin, C. T. (2007). Biodegradable polymers as biomaterials. *Progress in Polymer Ccience*, *32*(8), 762-798.

[113] Oliveira, M. B., Ribeiro, M. P., Miguel, S. P., Neto, A. I., Coutinho, P., Correia, I. J., & Mano, J. F. (2014). In vivo high-content evaluation of three-dimensional scaffolds biocompatibility. *Tissue Engineering Part C: Methods*, *20*(11), 851-864.

[114] Nilsson, B., Ekdahl, K. N., Mollnes, T. E., & Lambris, J. D. (2007). The role of complement in biomaterial-induced inflammation. *Molecular Immunology*, *44*(1), 82-94.

[115] Tang, L., & Hu, W. (2005). Molecular determinants of biocompatibility. *Expert review of medical devices*, *2*(4), 493-500.

[116] Gallagher, W. M., Lynch, I., Allen, L. T., Miller, I., Penney, S. C., O'Connor, D. P., et al. (2006). Molecular basis of cell–biomaterial interaction: insights gained from transcriptomic and proteomic studies. *Biomaterials*, *27*(35), 5871-5882.

[117] Kao, W. J., & Lee, D. (2001). In vivo modulation of host response and macrophage behavior by polymer networks grafted with fibronectin-derived biomimetic oligopeptides: the role of RGD and PHSRN domains. *Biomaterials*, *22*(21), 2901-2909.

[118] Anderson, J. M. (2001). Biological responses to materials. *Annual Review of Materials Research*, *31*(1), 81-110.

[119] Use of International Standard ISO-10993: Biological Evaluation of Medical Devices Part-1: Evaluation and Testing. Washington, DC: U.S. Food and Drug Administration, Department of Health and Human Services, 1995. Available at https://www.fda.gov/downloads/medicaldevices/deviceregulationandguidance/guidancedocuments/ucm348890.pdf (accessed October 8, 2017).

[120] Kirkpatrick, C. J., Krump-Konvalinkova, V., Unger, R. E., Bittinger, F., Otto, M., & Peters, K. (2002). Tissue response and biomaterial integration: the efficacy of in vitro methods. *Biomolecular Engineering*, *19*(2), 211-217.

[121] Anderson, J. M., Rodriguez, A., & Chang, D. T. (2008). Foreign body reaction to biomaterials. In *Seminars in Immunology* (Vol. 20, No. 2, pp. 86-100). Academic Press.

[122] Sheikh, Z., Brooks, P. J., Barzilay, O., Fine, N., & Glogauer, M. (2015). Macrophages, foreign body giant cells and their response to implantable biomaterials. *Materials*, *8*(9), 5671-5701.

[123] Anderson, J. M. (2015). Exploiting the inflammatory response on biomaterials research and development. *Journal of Materials Science: Materials in Medicine*, *26*(3), 121.

[124] Kanagaraja, S., Alaeddine, S., Eriksson, C., Lausmaa, J., Tengvall, P., Wennerberg, A., & Nygren, H. (1999). Surface characterization, protein adsorption, and initial cell–surface reactions on glutathione and 3-mercapto-1, 2,-propanediol immobilized to gold. *Journal of Biomedical Materials Research Part A*, *46*(4), 582-591.

[125] Gorbet, M. B., & Sefton, M. V. (2004). Biomaterial-associated thrombosis: roles of coagulation factors, complement, platelets and leukocytes. *Biomaterials*, *25*(26), 5681-5703.

[126] Andersson, J., Ekdahl, K. N., Larsson, R., Nilsson, U. R., & Nilsson, B. (2002). C3 adsorbed to a polymer surface can form an initiating alternative pathway convertase. *The Journal of Immunology*, *168*(11), 5786-5791.

[127] Brash, J. L. (1991). Role of plasma protein adsorption in the response of blood to foreign surfaces. In C. P. Sharma, & M. Szycher, *Blood Compatible Materials and Devices.* (pp. 3-24). Lancaster, PA, US: Technomic.

[128] Tang, L., & Eaton, J. W. (1995). Inflammatory responses to biomaterials. *American Journal of Clinical Pathology*, *103*(4), 466-471.

[129] Gray, J. J. (2004). The interaction of proteins with solid surfaces. *Current Opinion in Structural Biology*, *14*(1), 110-115.

[130] Pankowsky, D. A., Ziats, N. P., Topham, N. S., Ratnoff, O. D., & Anderson, J. M. (1990). Morphologic characteristics of adsorbed human plasma proteins on vascular grafts and biomaterials. *Journal of Vascular Surgery*, *11*(4), 599-606.

[131] Kottke-Marchant, K., Anderson, J. M., Umemura, Y., & Marchant, R. E. (1989). Effect of albumin coating on the in vitro blood compatibility of Dacron® arterial prostheses. *Biomaterials*, *10*(3), 147-155.

[132] Jenney, C. R., & Anderson, J. M. (2000). Adsorbed IgG: a potent adhesive substrate for human macrophages. *Journal of Biomedical Materials Research Part A*, *50*(3), 281-290.

[133] Tang, L., & Eaton, J. W. (1993). Fibrin (ogen) mediates acute inflammatory responses to biomaterials. *Journal of Experimental Medicine*, *178*(6), 2147-2156.

[134] Horbett, T. A. (1982). Protein adsorption on biomaterials. In S. L. Cooper, N. A. Peppas, A. S. Hoffman, & B. D. Ratner, *Biomaterials: Interfacial Phenomena and Applications* (Vol. 199, pp. 233–244). American Chemical Society.

[135] Wu, Y., Simonovsky, F. I., Ratner, B. D., & Horbett, T. A. (2005). The role of adsorbed fibrinogen in platelet adhesion to polyurethane surfaces: a comparison of surface hydrophobicity, protein adsorption, monoclonal antibody binding, and platelet adhesion. *Journal of Biomedical Materials Research Part A*, *74*(4), 722-738.

[136] Ariëns, R. A. S. (2013). Fibrin (ogen) and thrombotic disease. *Journal of Thrombosis and Haemostasis*, *11*(s1), 294-305.

[137] Herrmann, M., Vaudaux, P. E., Pittet, D., Auckenthaler, R., Lew, D. P., Schumacher-Perdreau, F., et al. (1988). Fibronectin, fibrinogen, and laminin act as mediators of adherence of clinical staphylococcal isolates to foreign material. *The Journal of Infectious Diseases*, *158*(4), 693-701.

[138] Anderson, J. M. (1988). Inflammatory response to implants. *ASAIO Journal*, *34*(2), 101-107.

[139] Babensee, J. E., Anderson, J. M., McIntire, L. V., & Mikos, A. G. (1998). Host response to tissue engineered devices. *Advanced drug delivery reviews*, *33*(1), 111-139.

[140] Anderson, J. M. (1993). Mechanisms of inflammation and infection with implanted devices. *Cardiovascular Pathology*, *2*(3), 33-41.

[141] Maluf-Meiken, L. C., Silva, D. R., Duek, E. A., & Alberto-Rincon, M. C. (2006). Morphometrical analysis of multinucleated giant cells in subdermal implants of poly-lactic acid in rats. *Journal of Materials Science: Materials in Medicine*, *17*, 481-485.

[142] Mainil-Varlet, P., Gogolewski, S., & Nieuwenhuis, P. (1996). Long-term soft tissue reaction to various polylactides and their in vivo degradation. *Journal of Materials Science: Materials in Medicine*, *7*(12), 713-721.

[143] Padial-Molina, M., Galindo-Moreno, P., Fernández-Barbero, J. E., O'Valle, F., Jódar-Reyes, A. B., Ortega-Vinuesa, J. L., & Ramón-Torregrosa, P. J. (2011). Role of wettability and nanoroughness on interactions between osteoblast and modified silicon surfaces. *Acta biomaterialia*, *7*(2), 771-778.

[144] Anselme, K. (2000). Osteoblast adhesion on biomaterials. *Biomaterials*, *21*(7), 667-681.

[145] Horbett, T. A. (1994). The role of adsorbed proteins in animal cell adhesion. *Colloids and Surfaces B: Biointerfaces*, *2*(1-3), 225-240.

[146] Hiraguchi, Y., Nagahashi, K., Shibayama, T., Hayashi, T., Yano, T. A., Kushiro, K., & Takai, M. (2014). Effect of the distribution of adsorbed proteins on cellular adhesion behaviors using surfaces of nanoscale phase-reversed amphiphilic block copolymers. *Acta biomaterialia*, *10*(7), 2988-2995.

[147] Ekdahl, K. N., Nilsson, B., Gölander, C. G., Elwing, H., Lassen, B., & Nilsson, U. R. (1993). Complement activation on radio frequency plasma modified polystyrene surfaces. *Journal of colloid and interface science*, *158*(1), 121-128.

[148] Athanasiou, K. A., Niederauer, G. G., & Agrawal, C. M. (1996). Sterilization, toxicity, biocompatibility and clinical applications of polylactic acid/polyglycolic acid copolymers. *Biomaterials*, *17*(2), 93-102.

[149] Song, W., Veiga, D. D., Custódio, C. A., & Mano, J. F. (2009). Bioinspired degradable substrates with extreme wettability properties. *Advanced Materials*, *21*(18), 1830-1834.

[150] Figueiredo, A., Coimbra, P., Cabrita, A., Guerra, F., & Figueiredo, M. (2013). Comparison of a xenogeneic and an alloplastic material used in dental implants in terms of physico-chemical characteristics and in vivo inflammatory response. *Materials Science and Engineering: C*, 33(6), 3506-3513.

[151] Kobayashi, S. D., Malachowa, N., & DeLeo, F. R. (2015). Pathogenesis of Staphylococcus aureus abscesses. *The American journal of pathology*, *185*(6), 1518-1527.

[152] Brodbeck, W. G., Patel, J., Voskerician, G., Christenson, E., Shive, M. S., Nakayama, Y., et al. (2002). Biomaterial adherent macrophage apoptosis is increased by hydrophilic and anionic substrates in vivo. *Proceedings of National Academy of Sciences*, *99*(16), 10287-10292.

[153] Shen, M., & Horbett, T. A. (2001). The effects of surface chemistry and adsorbed proteins on monocyte/macrophage adhesion to chemically modified polystyrene surfaces. *Journal of Biomedical Materials Research Part A*, *57*(3), 336-345.

[154] Tang, L., Thevenot, P., & Hu, W. (2008). Surface chemistry influences implant biocompatibility. *Current Topics in Medicinal Chemistry*, *8*(4), 270-280.

[155] Wang, Q. S., Cui, Y. L., Gao, L. N., Guo, Y., Li, R. X., & Zhang, X. Z. (2014). Reduction of the pro-inflammatory response by tetrandrine-loading poly (l-lactic acid) films in vitro and in vivo. *Journal of Biomedical Materials Research Part A*, *102*(11), 4098-4107.

[156] Goreish, H. H., Lewis, A. L., Rose, S., & Lloyd, A. W. (2004). The effect of phosphorylcholine-coated materials on the inflammatory response and fibrous capsule formation: In vitro and in vivo observations. *Journal of Biomedical Materials Research Part A*, *68*(1), 1-9.

[157] Rosa, A. L., Beloti, M. M., & van Noort, R. (2003). Osteoblastic differentiation of cultured rat bone marrow cells on hydroxyapatite with different surface topography. *Dental materials*, *19*(8), 768-772.

[158] Ranella, A., Barberoglou, M., Bakogianni, S., Fotakis, C., & Stratakis, E. (2010). Tuning cell adhesion by controlling the roughness and wettability of 3D micro/nano silicon structures. *Acta biomaterialia*, *6*(7), 2711-2720.

[159] Recum, A. F. V., Shannon, C. E., Cannon, C. E., Long, K. J., Kooten, T. G. V., & Meyle, J. (1996). Surface roughness, porosity, and texture as modifiers of cellular adhesion. *Tissue Engineering*, *2*(4), 241-253.

[160] Song, W., & Mano, J. F. (2013). Interactions between cells or proteins and surfaces exhibiting extreme wettabilities. *Soft Matter*, *9*(11), 2985-2999.

[161] Kim, M. S., Ahn, H. H., Shin, Y. N., Cho, M. H., Khang, G., & Lee, H. B. (2007). An in vivo study of the host tissue response to subcutaneous implantation of PLGA-and/or porcine small intestinal submucosa-based scaffolds. *Biomaterials*, *28*(34), 5137-5143.

[162] Wang, Z., Wang, Y., Ito, Y., Zhang, P., & Chen, X. (2016). A comparative study on the in vivo degradation of poly (L-lactide) based composite implants for bone fracture fixation. *Scientific Reports*, *6*.

[163] Bos, R. R., Rozema, F. R., Boering, G., Nijenhuis, A. J., Pennings, A. J., Verwey, A. B., et al. (1991). egradation of and tissue reaction to biodegradable poly (L-lactide) for use as internal fixation of fractures: a study in rats. *Biomaterials*, *12*(1), 32-36.

[164] Midwood, K. S., Williams, L. V., & Schwarzbauer, J. E. (2004). Tissue repair and the dynamics of the extracellular matrix. *The International Journal of Biochemistry & Cell Biology*, *36*(6), 1031-1037.

[165] Andersson, M., Suska, F., Johansson, A., Berglin, M., Emanuelsson, L., Elwing, H., & Thomsen, P. (2008). Effect of molecular mobility of polymeric implants on soft tissue reactions: an in vivo study in rats. *Journal of Biomedical Materials Research Part A*, *84*(3), 652-660.

[166] Grayson, A. C., Voskerician, G., Lynn, A., Anderson, J. M., Cima, M. J., & Langer, R. (2004). Differential degradation rates in vivo and in vitro of biocompatible poly (lactic acid) and poly (glycolic acid) homo-and co-polymers for a polymeric drug-delivery microchip. *Journal of Biomaterials Science, Polymer Edition*, *15*(10), 1281-1304.

[167] Suska, F., Emanuelsson, L., Johansson, A., Tengvall, P., & Thomsen, P. (2008). Fibrous capsule formation around titanium and copper. *Journal of Biomedical Materials Research Part A*, *85*(4), 888-896.

[168] Biau, D. J., Kernéis, S., & Porcher, R. (2008). Statistics in brief: the importance of sample size in the planning and interpretation of medical research. *Clinical Orthopaedics and Related Research*, *466*(9), 2282-2288.

[169] Bauer, T. W., & Mahovlic, D. (2003). Cutting and grinding methods for hard-tissue histology. In *Handbook of Histology Methods for Bone and Cartilage* (pp. 233-242). Humana Press.

[170] Engqvist, H., Botton, G. A., Couillard, M., Mohammadi, S., Malmström, J., Emanuelsson, L., et al. (2006). A novel tool for high-resolution transmission electron microscopy of intact interfaces between bone and metallic implants. *Journal of Biomedical Materials Research Part A, 78*(1), 20-24.

[171] Chai, W. L., Moharamzadeh, K., Brook, I. M., & Van Noort, R. (2011). A review of histomorphometric analysis techniques for assessing implant-soft tissue interface. *Biotechnic & Histochemistry*, *86*(4), 242-254.

[172] Vacanti, N. M., Cheng, H., Hill, P. S., Guerreiro, J. D., Dang, T. T., Ma, M., et al. (2012). Localized delivery of dexamethasone from electrospun fibers reduces the foreign body response. *Biomacromolecules, 13*(10), 3031-3038.