MODIFICATION OF POLYMERIC PARTICLES VIA SURFACE GRAFTING FOR 3D SCAFFOLD DESIGN

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AKADEMISK AVHANDLING

Som med tillstånd av Kungliga Tekniska Högskolan i Stockholm framlägges till offentlig granskning för avläggande av teknisk doktorsexamen torsdagen den 29 oktober 2015, kl. 10.00 i sal E3, Osquarsbacke 14, våning 5, KTH, Stockholm. Avhandlingen förvaras på engelska.
In memory of my father

Yohanes Leonardus Sarminto

’’Akulah Jalan, Kebenaran dan Hidup. Tidak ada seorangpun yang datang kepada Bapa, kalau tidak melalui Aku’’ (Yoh 14:6)

Dedicated to my dearest mother
ABSTRACT

Surface modification techniques have played important roles in various aspects of modern technology. They have been employed to improve substrates by altering surface physicochemical properties. An ideal surface modifying technique would be a method that is applicable to any kind of materials prepared from a wide range of polymers and that can occur under mild reaction conditions. The work in this thesis has utilized four main concepts: I) the development of a ‘grafting-from’ technique by covalently growing polymer grafts from particle surfaces, II) the presence of steric and electrosteric forces due to long-range repulsive interactions between particles, III) a combined surface grafting and layer-by-layer approach to create polyelectrolyte multilayers (PEMs) on particle surfaces to fabricate strong and functional materials, and IV) the roles of hydrophilic polymer grafts and substrate geometry on surface degradation.

A non-destructive surface grafting technique was developed and applied to polylactide (PLA) particle surfaces. Their successful modification was verified by observed changes to the surface chemistry, morphology and topography of the particles. To quantify the aggregation behavior of grafted and non-grafted particles, force interaction measurements were performed using colloidal probe atomic force microscopy (AFM). Long-range repulsive interactions were observed when symmetric systems, i.e., hydrophilic polymer grafts on two interacting surfaces, and asymmetric system were applied. Electrosteric forces were observed when the symmetric substrates interacted at pH 7.4. When PEMs were alternately assembled on the surface of poly(L-lactide) (PLLA) particles, the grafted surfaces played a dominated role in altering the surface chemistry and morphology of the particles. Three-dimensional scaffolds of surface grafted particle coated with PEMs demonstrated high mechanical performance that agreed well with the mechanical performance of cancellous bone. Nanomaterials were used to functionalize the scaffolds and further influence their physicochemical properties. For example, when magnetic nanoparticles were used to functionalize the scaffolds, a high electrical conductivity was imparted, which is important for bone tissue regeneration. Furthermore, the stability of the surface grafted particles was evaluated in phosphate buffered saline (PBS) solution. The nature of the hydrophilic polymer grafts and the geometry of the PLLA substrates played central roles in altering the surface properties of films and particles. After 10 days of PBS immersion, larger alterations in the surface morphology were observed on the film compared with microparticles.
grafted with poly(acrylic acid) (PAA). In contrast to the PAA-grafted substrates, the morphology of poly(acrylamide) (PAAm)-grafted substrates was not affected by PBS immersion. Additionally, PAAm-grafted microparticulate substrates encountered surface degradation more rapidly than PAAm-grafted film substrates.

**Keywords**: surface grafting, PLA, PLLA, hydrophilic polymers, particles, geometry, steric stabilization, atomic force microscopy (AFM), polyelectrolyte multilayers, 3D scaffold, bone tissue engineering, surface degradation
SAMMANFATTNING

Ytmodifiering spelar idag en viktig roll i ett stort antal olika aspekter av modern teknologi, eftersom ytmodifiering används för att förbättra ytans fysikaliska och kemiska egenskaper. En optimal ytmodifieringsteknik kan appliceras på ett brett spektrum av substrat utan att materialets övriga egenskaper förändras. Denna avhandling har baserats på fyra huvudkoncept: I) utveckling av en "ympning-från"-teknik genom vilken kovalent bundna polymerer ympas på ytan hos PLA partiklar utan att partiklarnas bulkegenskaper förändras, II) design av dessa partiklars ytor för att förhindra agglomerering genom att repulsiva interactioner (steriska- och elektrosteriska krafter) skapas mellan partiklarna, III) utveckling av en kombinerad ytymning och skikt-för-skiktmетод för att tillverka ett polyelektrolytmaterial (PEM) på partiklarna och på så sätt tillverka starka och funktionella material, och IV) betydelsen av den kemiska strukturen hos de hydrofila ympolymerer och geometrin hos substraten vid ytndöd.
polymerer i PBS. Däremot kunde man se en snabbare nedbrytning av ytan hos partiklarna jämfört med filmerna när de var ympade med PAAm.

**Nyckelord:** ytymning, PLA, PLLA, hydrofila polymerer, partiklar, geometri, sterisk stabilisering, atomkraftsmikroskopi (AFM), polyelektrolytmultilager, 3D scaffolds, benregenerering, ytnedbrytning
This thesis is a summary of the following papers:


The contributions of the author to the appended papers:

I. All of the experimental work and data evaluation, and much of the preparation of the manuscript.
II. All of the experimental work and data evaluation, and major parts of the preparation of the manuscript.
III. All of the experimental work and data evaluation, and major parts of the preparation of the manuscript.
IV. All of the experimental work (except fluorescence microscopy, porosity, electrical conductivity, nanomaterials preparation) and data evaluation, and major parts of the preparation of the manuscript.

Papers not included in the thesis:


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ABBREVIATIONS

3D  Three-dimensional
A   Absorbance
AA  Acrylic Acid
AAm Acrylamide
AdG Alexander-de Gennes
AFM Atomic Force Microscopy
ATR Attenuated Total Reflectance
BP Benzophenone
D   Distance
Ð   Dispersity
ΔG Change in Gibbs energy
ΔH Change in enthalpy
ΔS Change in entropy
DCM Dichloromethane
DLVO Derjaguin-Landau-Verwey-Overbeek
EDL Electrical Double Layer
F   Force
Fe3O4 Ferric Oxide
FTIR Fourier Transform Infra Red
GO  Graphene Oxide
Γ   Grafting density
Lo  Thickness
LbL Layer-by-Layer
MAH Maleic Anhydride
µCT Micro-CT
Mn  Number-average molecular weight
O/W Oil-in-Water
PAA Poly(acrylic acid)
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<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>PAAm</td>
<td>Poly(acrylamide)</td>
</tr>
<tr>
<td>PAH</td>
<td>Poly(allylamine hydrochloride)</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate Buffered Saline</td>
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<td>PEMs</td>
<td>Polyelectrolyte Multilayers</td>
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<td>PLA</td>
<td>Polylactide</td>
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<td>PLA-g-PMAH</td>
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<td>PMAH</td>
<td>Poly(maleic anhydride)</td>
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<td>R</td>
<td>Radius</td>
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<td>RO</td>
<td>Reverse Osmosis</td>
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<td>s</td>
<td>Average distance of grafting site</td>
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<td>SEC</td>
<td>Size Exclusion Chromatography</td>
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<td>UV</td>
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1 PURPOSE OF THE STUDY

Surface modification via surface grafting constitutes a technique used to improve the physicochemical properties of various materials, such as films, fibers and particles. Surface grafting can be used to create high-density grafting layers by the covalent attachment of polymer grafts to the substrates surfaces. One reason to modify particle surfaces is to use the covalently grafted layers to overcome particle aggregation, as particle aggregation has limited the use of particles for a broad spectrum of applications. Furthermore, surface grafting can also be used to build attractive forces on the particle surfaces, as is the case for the assembly of polyelectrolyte multilayers (PEMs). This strategy could enable the fabrication of highly functional particle-based scaffolds and have applications in tissue engineering. To reach long term stability of the polymer grafts, the risk of only temporary attachment of the polymer grafts on different substrate surfaces needs to be controlled. In other words, the stability issue in the polymer grafts is an important focus that needs to be evaluated. Therefore, the goal of this work is to:

- design a non-destructive ‘grafting-from’ technique applicable to PLA particle surfaces and thereby induce alterations in the surface chemistry, morphology and topography (Paper I);
- control the steric stabilization of PLA particles through long-range repulsive interactions (Paper II);
- combine surface grafting and layer-by-layer (LbL) techniques to fabricate 3D PLLA microsphere scaffolds (Paper IV); and
- stress the roles of polymer grafts and PLLA substrate geometry on the early stages of surface degradation (Paper III).
2 INTRODUCTION

2.1 BACKGROUND

Favorable bulk properties, such as mechanical performance, solvent resistance, durability, and thermal stability, are reasons why a polymeric material is generally selected for a specific application. However, the selected polymer could have surface characteristics, e.g., hydrophobicity, chemical inertness, non-polar groups, that are not desired or even detrimental for the intended application. To overcome these problems, strategies dealing with surface modification have been developed and implemented. Surface modification techniques can be divided into two broad groups:

- physical-based surface modifications based on adsorption on existing surfaces to produce new surface compositions (e.g., self-assembly and layer-by-layer and Langmuir-Blodgett techniques); and
- chemical-based surface modifications based on covalently attachment to existing surfaces to produce new surface compositions (e.g., UV-irradiation grafting and alkaline hydrolysis).

Thus, surface modification techniques and surface interaction regulations of polymers with other materials have been of significant interest in applications where polymeric materials are used. A few of these techniques have been used to introduce new functional groups on the polymer surfaces to induce changes in, e.g., surface morphology, hardness, adhesiveness, roughness, conductivity, and lubrication. While a theoretical distinction is recognized between material surface and bulk properties, for the purposes of surface modifications, only the outermost surface is typically considered relevant. Various techniques for chemical-based surface modifications have been used to change aliphatic polyester surfaces for uses in such applications as ozone treatment, vapor phase grafting, high-energy irradiation, alkaline hydrolysis reactions, and plasma treatments. A few techniques have been
proposed to use UV-irradiation to produce polymeric materials with new functionalities.\textsuperscript{2,17-19}

### 2.2 ‘GRAFTING-FROM’ POLYMERIZATION

Surface modification via UV-irradiation, or photoinitiated polymerization, has attracted interest ranging from conventional applications in microelectronics, optical waveguides, coatings, inks, and adhesives, to 3D biomaterials for tissue engineering.\textsuperscript{19-24} Polymer chains of functional groups can be covalently attached to the substrate surface by two different approaches: ‘grafting-from’ and ‘grafting-to’.\textsuperscript{25} The former refers to the synthesis of polymer chains from monomers via polymerization with photoinitiators, whereas the latter refers the attachment of fully synthesized polymers to the substrate surface.\textsuperscript{3} The ‘grafting-from’ technique is more appropriate when a thicker, higher grafting density polymer film is desired because of fewer diffusion-related issues and lower steric hindrances commonly encountered with the ‘grafting-to’ technique.\textsuperscript{26,27} Both techniques result in the covalent attachment of functional polymer chains to the substrate surface. A commonly-used and versatile method to attach unsaturated monomers, i.e., vinyl or acrylic monomers, is by free-radical polymerization. When modifying the surface via free-radical polymerization, unpaired electrons are formed on the surface of interest, thereby making the surface chemically reactive. However, care must be taken to only limiting the reactive sites to the surface of the material and not the bulk. Unpaired electrons can also be produced on the substrate surface by several methods including UV-irradiation.\textsuperscript{3} UV-irradiation is an attractive method for graft polymerization due to its cleanliness, simplicity, low energy cost, and permanent covalent attachment.\textsuperscript{2}

### 2.3 COLLOIDAL STABILITY OF GRAFTED POLYMERS

One of the methods used to prevent the aggregation of colloidal particles is to bond polymeric layers onto the surfaces of the particles. Photografted polymer chains act as protective layers preventing particle aggregation due to van der Waals forces.\textsuperscript{28,29} The chains are also sterically stabilized due to a positive Gibbs free energy: \( \Delta G = \Delta H - T\Delta S \), where a negative \( \Delta G \) indicates particle aggregation while a positive \( \Delta G \) induces stable particle dispersion. If a good solvent is used as a medium for stabilizing the chains, the grafting layers anchored on the colloidal particle surfaces have difficulty interpenetrating each other when they approach and instead collide. This phenomenon decreases the number of configurations available to the anchored polymer chains and leads to a negative entropy change and eventually a positive \( \Delta G \). However, if a poor solvent medium is used, the grafting layers are thermodynamically favored to interpenetrate and induce aggregation.\textsuperscript{30}
2.3.1 The DLVO and AdG models

The Derjaguin-Landau-Verwey-Overbeek (DLVO) and the Alexander-de Gennes (AdG) models are two scientific concepts describing electrostatic and steric mechanisms, respectively. The former model explains how electrostatic interactions stabilize colloids, while the latter shows how steric forces play dominant roles in overcoming particle aggregation. The DLVO theory predicts that repulsive Coulombic interactions at large separations are observed when highly charged colloidal particles interact. This theory provides an approximate solution to the Poisson-Boltzmann equation in describing the total coupling between two interaction forces, i.e., van der Waals forces and repulsive electrostatic forces, according to the following equations:

\[ V_{\text{tot}} = V_{\text{electrostatic}} + V_{\text{vdW}} \]  
\[ V_{\text{tot}} = \left( \frac{64\pi k T \rho \infty \gamma^2}{\kappa^2} \right) e^{-\kappa D} + \frac{-AR}{6D} \]

where \( k \) is the Boltzmann constant, \( T \) is the temperature, \( R \) is the radius, \( \rho \infty \) is the ionic concentration of ions in the bulk, \( \phi_0 \) is surface potential, \( 1/\kappa \) is the Debye length, \( D \) is the separation distance, \( A \) is the Hamaker constant, and \( \gamma \) can be calculated from \( \gamma = \tanh \left( \frac{\phi_0 (\text{mV})}{103} \right) \). \( V_{\text{vdW}} \) is dependent on the particle size, the Hamaker constant, and the distance between the particles, while \( V_{\text{electrostatic}} \) is affected by the zeta potential, particle size, ion concentration, distance between particles, and medium dielectric constant. Due to the screening of the surface charge, the electrical double layer (EDL) decreases as the ionic strength increases in the medium. This phenomenon causes a decrease in \( V_{\text{electrostatic}} \) and thereby, results in the tendency of the particles to agglomerate. Repulsive forces are produced when particle EDLs overlap in the aqueous medium due to electrostatic interaction. As a consequence, particle agglomeration can be avoided. The EDL consists of two main layers: (1) the Stern layer, which comprises counter ions electrostatically attracted toward the surface of oppositely charged particles (thus, maintaining the electroneutrality of the aqueous system) and (2) the Gouy layer, which is filled with a diffusion layer of ions (Figure 1).
Figure 1. An illustration of EDL surrounding a negatively charged particle and consisting of a (A) Stern layer and a (B) Gouy layer.

Another mathematical model that can be used to highlight the phenomenon of steric-stabilization is the Alexander-de Gennes (AdG) model.36 This model assumes that all polymer chains are uncharged and no interpenetration or adsorption occurs between polymer grafts. This model, also called the box-model, assumes that the grafted chains take the form of polymer brushes with blobs (Figure 2). Furthermore, the model assumes that the polymer grafts are uniformly stretched out and that all chain ends are placed at the edge of the polymer brush. This confinement of the polymer grafts leads to the formation of a number of blobs per chain due to the influence of other neighboring polymer grafts, as displayed in Figure 2.
Figure 2. An illustration of the polymer brush from the AdG model. The size of polymer blob (ξ) is calculated from the average distance between two grafting sites (s) of the polymer graft.

A blob of the polymer graft is defined as a string of monomers, the conformation of which is not perturbed by any intermolecular interactions. De Gennes later applied these ideas to describe the interaction force per unit area between two parallel plates covered by polymer grafts:

\[
f(D) = \frac{\pi B}{s^3} [(\frac{2L_o}{D})^\frac{9}{4} - (\frac{D}{2L_o})^{\frac{3}{4}}] \quad D < 2L_o
\]

\[
\Gamma = \frac{1}{s^2}
\]

where \( f(D) \) is the interaction per unit area, \( k_B \) is Boltzmann’s constant, \( T \) is the temperature, \( s \) is the average distance of grafting site, \( L_o \) is the thickness of the polymer graft, \( D \) is the distance between near substrates, and \( \Gamma \) is the grafting density.

2.4 THE ASSEMBLY OF POLYELECTROLYTE MULTILAYERS (PEMS)

Layer-by-layer (LbL) is a surface deposition technique used in template-assisted assembly. This technique was initially used to prepare structure-controlled thin films in biological applications. This method is quite simple because many surfaces, such as glasses, metals, silicons, and polymers, have net negative charges in solutions due to hydrolysis and surface oxidation (Figure 3A). When one of these materials is immersed in a positively charged solution of polyelectrolytes, such as poly(ethylene imine) (PEI) or poly(allylamine hydrochloride) (PAH), followed by pure water rinsing, the net surface charge becomes positive due to the physical adsorption and charge overcompensation carried by the polyelectrolytes (Figure 3B).
**Introduction**

Figure 3. The adsorption of polyelectrolyte multilayers on a film surface.

The subsequent deposition of a negatively charged polyelectrolyte solution, such as poly(acrylic acid) (PAA) or poly(styrene sulfonate) (PSS), results in a net charge reversal on the substrate surface (Figure 3C). A bilayer of polyelectrolytes is thereby produced on the substrate surface. With such alternating surface treatments, polyelectrolyte multilayers (PEMs) with controllable thickness and structure can be obtained.\(^{43}\) When compared with self-assembled monolayers (SAMs) and Langmuir-Blodgett surface modification techniques, the structure of the PEMs of thin films fabricated via the LbL technique provides higher loading and allows for more stable films.\(^{44}\)

The use of the LbL technique has numerous benefits. LbL can be performed in aqueous solutions, i.e., without any unnecessary exposure to organic solvents. This is especially applicable for preparations involving biomacromolecules and biomolecules, such as proteins and nucleic acids, because they are sometimes difficult to dissolve in organic solvents and are easily denatured. This process is also relatively cheap, requiring simple laboratory equipment and can be carried out with inexpensive reagents.\(^{45}\) From these viewpoints, LbL assembly is a promising technique and has been considered in preparing devices in varied fields, such as electronics,\(^ {46,47}\) optoelectronics,\(^ {48,49}\) and therapeutics.\(^ {50}\)

### 2.5 SCAFFOLD FABRICATION

In tissue engineering, three-dimensional (3D) scaffolds are often used as a temporary support for the regeneration of new cell tissue. The 3D scaffolds should most often degrade when the support is no longer needed. To support cellular activities, ideal 3D scaffolds should fulfill several requirements, including biocompatibility, biodegradability with nontoxic degradation products, porosity, and having suitable mechanical properties.\(^ {51}\)
For bone tissue engineering, four types of scaffolding material are commonly used: natural materials, bioceramics, inorganic metals, and synthetic polymers. The 3D polymeric scaffolds can be fabricated using a variety of techniques, such as solvent casting/salt leaching, chemical foaming, 3D printing, freeze drying, and microparticulate-based fabrication, which is usually achieved via a sintering at medium to high temperatures.

The mechanical properties of 3D scaffolds play important roles in tissue regeneration. For example, in bone tissue engineering, the mechanical performance of the 3D scaffolds should be within the range of the compressive modulus of cancellous bone, i.e., between 10-2000 MPa, to support tissue regeneration. Additionally, electric signals and correspondingly, conductive materials, can also be used to stimulate new bone formation.

2.6 SURFACE DEGRADATION

Surface erosion is one potential mechanism of polymer degradation. Degradation involves chain scissions wherein polymer chains undergo cleavage to form oligomers and then monomers. Erosion can be defined as the loss of monomers or oligomers leaving the bulk polymer. While polymer chain erosion occurs at a constant rate, this is not the case with bulk degradation, which usually occurs with no significant initial mass losses, followed by rapid mass losses.

There are a number of degradation mechanism in which the chain cleavage of degradable polymers is the driving force. Furthermore, a number of identified parameters affect the degradation process, such as molar mass, crystallinity, autocatalysis, copolymer compositions, the presence of other excipients or proteolytic drugs, pH and hydrophobicity.
Figure 4. Schematics of surface degradation and bulk degradation from an initial time period \((t=0)\) to later time periods \((t=t_n)\)

Surface-degrading particles lose materials from the surface as they become smaller in size but maintain their original shape (Figure 4). Surface erosion impacts such properties as structural morphology and the diffusion of monomers and oligomers. Understanding the erosion process on the surface of polymers plays an important role for the successful application of biodegradable polymers.\textsuperscript{65} For tissue engineering, the porosity and surface properties of 3D polymeric scaffolds affect their performance.\textsuperscript{79}
3 EXPERIMENTAL

3.1 MATERIALS

Two types of lactide-based polymers were used in this study, commercial PLA (Nature Works Co., Ltd., USA, grade 5200D, $M_n = 150,000$ g/mol, $\mathcal{D} = 1.50$) and synthesized PLLA ($M_n = 120,000$ g/mol, $\mathcal{D} = 1.20$ and $M_n = 50,000$ g/mol, $\mathcal{D} = 1.32$). PLLA was synthesized by the ring-opening polymerization (ROP) of L-lactide (LLA, Boehringer Ingelheim, Germany) at 110°C for 72 h in bulk, using ethylene glycol (Aldrich, Germany) as the initiator and stannous octoate ($\text{Sn(Oct)}_2$) (Sigma Aldrich, Sweden) as the catalyst. Benzophenone (BP) (99 %, Sigma Aldrich), acrylamide (AAm) (98.5 %, Acros), maleic anhydride (MAH) (> 99 %, Fluka), phosphate buffered saline (PBS) (VWR), hydrogen chloride (HCl) (2 M, Fisher Scientific), sodium hydroxide (NaOH) (99 %, Merck), chloroform (> 99 %, Fisher Scientific), graphene oxide (GO) (4-10 % edge oxidized, Sigma Aldrich). Acrylic acid (AA) (90%, Alfa Aesar) was purified by vacuum distillation at 40°C prior to use. Dichloromethane (DCM) (> 99 %, Fisher Scientific), ethanol (96 % v/v, VWR), polysorbate 80 (commonly known as Tween 80) (Fluka), three different weak polyelectrolytes, poly(acrylic acid) (PAA) (99 %, Sigma Aldrich), poly(allylamine hydrochloride) (PAH) (99 %, Alfa Aesar) and poly(acrylamide) (PAAm) (99 %, Polysciences), and sodium chloride (NaCl) (p.a., Merck) were used as received. Three different concentrations of NaCl solution, 0.1, 1 and 10 mM, were prepared using water pre-treated and connected to a Milli-RO unit (Merck, Darmstadt, Germany). A purification step was then carried out with a Milli-Q Plus 185 system. The water resistivity was 18.2 MΩ cm, and the pH of the Milli-Q water was measured and estimated to be approximately 5.6.
3.2 PARTICLE AND FILM FABRICATION

Oil-in-Water Emulsion. The emulsification technique, i.e., oil-in-water (O/W), was used to fabricate PLA microspheres. A 0.2 g sample of PLA pellets was dissolved in 100 mL of organic solvent, i.e., DCM, acting as the organic phase. Later, 10 mL of this organic phase was poured into 0.05 % w/v Tween 80 in aqueous solution acting as the continuous phase. This immiscible mixture was vigorously stirred overnight at room temperature until the organic solvent evaporated. The PLA microspheres finally settled at the bottom of the beaker and were found to have diameters in the range of 10-60 µm. The microsphere solution was filtered using a filter paper to recover the microspheres, which were washed several times with deionized water to remove residual surfactant and dried overnight.

Cryogenic Milling. A 10 g sample of PLA pellets was initially frozen in a cylindrical thermos filled with liquid nitrogen prevent overheating during milling. Mechanical grinding was carried out using a ZM 200 Retsch mill in a stainless steel bath; the rotational velocity was adjusted to approximately 12,000 rpm. After one minute of mechanical grinding, irregular PLA particles were recovered having a Feret’s diameter range of 600-1200 µm. As previously noted, the milling process was run for a maximum time of 1 min to prevent thermal degradation.

Spray drying technique. A 1% (w/v) solution of PLLA in chloroform was prepared. PLLA microparticles were then fabricated using a spray dryer (Mini Spray Dryer BUCHI B-290, Flawill, Switzerland). The following system parameters were used: inlet temperature (T_inlet) 55 - 65°C, aspirator 100% (35 m³/min), pump feed rate 20%, gas flow rate 50 mm (750 Nl/h). The dried microparticles with diameter in the range of 2-5 µm were recovered in a collecting device located beneath the cyclone of the spray dryer.

Solvent Casting. A 4 g sample of PLA and PLLA was dissolved in 100 mL chloroform. The solution was poured into a glass Petri dish, and the solvent was allowed to slowly evaporate. For force measurements, 2-cm wide, 1-cm long rectangular pieces were cut from the circular film and used as sample substrates. For surface degradation experiments, PLLA films were punched to obtain uniform samples with an approximate mass of 22 ± 2 mg and a 1-cm diameter, 0.22-mm thick circular shape.
3.3 SURFACE GRAFTING VIA ‘GRAFTING-FROM’ TECHNIQUE

Photoactivation. A 5% w/v solution of BP in ethanol was poured into a Pyrex tube containing particles later to be used to activate the inert surface of the PLA particles. The solution containing the PLA particles was then placed on a magnetic stirring plate and UV-irradiated for 20 min at 280-320 nm. The activated particles were centrifuged using a Hettich Universal 30 at 5000 rpm for 3 min to remove unreacted photoinitiator. Then, the activated particles were rinsed with ethanol several times. Afterward, they were placed in a closed Petri dish, dried overnight at room temperature, and finally characterized by FTIR spectroscopy. Control particles were also prepared using the same procedure but without photoinitiator. PLA films were also activated in the same way as the particles.

Photoinduced Polymerization of the activated PLA, PLLA particles and PLA, PLLA films. The surface-activated particles were polymerized with 20% w/v acrylamide (AAm), 20% w/v acrylic acid (AA) or 20% v/v maleic anhydride (MAH). The activated particles were placed in Pyrex tubes and ethanol was added. The system was then UV-irradiated for different periods of 15 min, 30 min, 45 min, 60 min (1 h), and 90 min (1.5 h). To remove all non-grafted polymer chains, the surface-grafted particles were spun down at 5000 rpm for 3 min, rinsed with ethanol, dried overnight, and characterized by FTIR spectroscopy. Control particles were also prepared using the same procedure without hydrophilic monomer. For the PLA films used in force measurements, two monomers, 20% w/v AAm and 20% v/v AA, were used, and the particles were exposed to UV-irradiation for 90 min. For the PLLA films and microparticles in the surface degradation experiments, AA, AAm, and an AA/AAm mix (1:1 v/v) were used. The samples were UV-irradiated for 90 min. Prior to the LbL assembly of the polyelectrolyte multilayers, the BP-activated PLLA microparticles were surface grafted with 20% v/v AA under UV-irradiation for 90 min.

3.4 FABRICATION OF THE 3D SCAFFOLD

The assembly of polyelectrolyte multilayers (PEMs). The polyelectrolyte multilayers (PEMs) were assembled onto the surfaces of substrate as described elsewhere. The method utilizes hydrogen bonding interactions between anionic (PAA) and neutral polymer (PAAm) polyelectrolytes at pH 2.5, denoted as PAA/PAAm, or electrostatic interactions between anionic (PAA) and cationic polymeric (PAH) polyelectrolytes at pH 2.5 and 5, denoted as PAA/PAH. Both layers were alternately deposited onto AA-grafted PLLA microspheres. Briefly, the particles were immersed in a 0.3 M polyelectrolyte solution (based on the repeating unit molecular weight) in 18.2 MΩ
cm MilliQ water. The system was vigorously stirred for approximately 15 min followed by centrifugation at 4000 rpm for 3 min. Water was used to wash away residual, unabsorbed polyelectrolytes in the solution between each adsorption cycle. Then, an adsorption, centrifugation, rinse, centrifugation cycle was conducted. The pH of the rinsing water was adjusted to the pH of the polyelectrolyte solutions, i.e., 2.5 and 5. To the system with the outermost layer of PAAm or PAH at pH 2.5, 0.002 wt. % of a functional additive, i.e., graphene oxide (GO) or iron oxide (Fe₃O₄) nanomaterials, was then alternately assembled onto the previously grafted particle surfaces. Non-grafted PLLA microspheres were also prepared using the same LbL procedure (Table 1).

Table 1. PAA-grafted PLLA microspheres used during the assembly of the polyelectrolyte multilayers and their respective references.

<table>
<thead>
<tr>
<th>Particulate substrate</th>
<th>Type of multilayer build-up</th>
<th>pH</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-grafted PLLA</td>
<td>PAA/PAAm</td>
<td>2.5</td>
<td>PAMₙ</td>
</tr>
<tr>
<td></td>
<td>PAA/PAH</td>
<td>2.5</td>
<td>PAH2.₅ₙ</td>
</tr>
<tr>
<td></td>
<td>PAA/PAH</td>
<td>5</td>
<td>PAH2.₅ₙ</td>
</tr>
<tr>
<td>PAA-grafted PLLA</td>
<td>PAA/PAAm</td>
<td>2.5</td>
<td>PAMₙ</td>
</tr>
<tr>
<td></td>
<td>PAA/PAH</td>
<td>2.5</td>
<td>PAH2.₅ₙ</td>
</tr>
<tr>
<td></td>
<td>PAA/PAH</td>
<td>5</td>
<td>PAH₅ₙ</td>
</tr>
<tr>
<td></td>
<td>PAA/PAAm/GO</td>
<td>2.5</td>
<td>PAMₙGO</td>
</tr>
<tr>
<td></td>
<td>PAA/PAH/GO</td>
<td>2.5</td>
<td>PAH₉GO</td>
</tr>
<tr>
<td></td>
<td>PAA/PAAm/Fe₃O₄</td>
<td>2.5</td>
<td>PAM₉FeO</td>
</tr>
<tr>
<td></td>
<td>PAA/PAH/Fe₃O₄</td>
<td>2.5</td>
<td>PAH₉FeO</td>
</tr>
</tbody>
</table>

3D scaffold fabrication. The polyelectrolyte multilayer-coated PLLA microspheres with carboxylic acids of PAA on the outermost layers were mixed with particles having PAAm on the outermost amide layers. The mix was rinsed with water and centrifuged. A similar procedure was performed for the electrostatic-driven samples,
i.e., particles that had PAA or PAH as the outermost layers. They were subsequently packed into a cylindrically shaped cut-off plastic syringe with a diameter range of 8-10 mm and a height range of 4-8 mm. The 3D microsphere scaffolds were dried for a week prior to characterization.

3.5 SURFACE DEGRADATION STUDIES

For samples containing PLLA microparticles, a predetermined amount (approximately 20-25 mg) of surface-grafted and neat microparticles as a reference were placed in a dialysis bag. Both types of samples, i.e., the PLLA films in circular shapes and dialysis bags containing PLLA microparticles, were later placed in 20 mL glass vials filled with 10 mL of PBS solution at pH 7.4 and then tightly closed with septa. The vials were put in a controlled incubator at 37 °C and shaken at rotational velocity of 60 rpm. At predetermined time intervals (5, 10, 14, and 30 days); both types of samples were taken out of the shaking incubator. The samples were finally dried under vacuum until they maintained their constant mass and were characterized subsequently.

3.6 CHARACTERIZATION METHODS

3.6.1 Size Exclusion Chromatography (SEC)

The molecular weights of the neat, control, activated, and grafted PLA and PLLA substrates were determined using a Waters 717 plus autosampler and a Waters model 510 apparatus equipped with two PL gel 10 µm mixed B columns, 300 x 7.5 mm (Polymer Laboratories, U.K). Chloroform was used as an eluent at a flow rate of 0.1 mL/min. The instrument was calibrated with polystyrene standards with narrow molecular weight distributions from 580 to 400,000 g/mol.

3.6.2 Fourier Transform Infrared (FTIR) Spectroscopy

The FTIR spectra of the neat PLA particles, neat PLLA films and microparticles, surface-grafted particles and films, polyelectrolyte-coated particles, and surface-degraded particles and films were recorded from 4000-600 cm⁻¹ on a Spectrum 2000 Perkin-Elmer spectrometer. An attenuated total reflectance (ATR) accessory (Golden Gate) provided analyses of sample surfaces to a depth of approximately 1 µm. All FTIR spectra were reported as the means of 5 samples and 16 individual scans at 4 cm⁻¹ resolution.
3.6.3 X-Ray Diffraction (XRD)

The crystalline structure of the GO-functionalized materials and their respective references were evaluated by X-ray diffraction (XRD) using a PANalytical Xpert Pro instrument with CuKα radiation at wavelength (λ) of 1.54 Å resulted from voltage and electric current of 45 kV and 45 mA, respectively. The diffractograms were recorded at temperature of 25°C using a silicon monocrystal sample holder at a step size angle of 0.017°. The intensity in the diffractogram was measured as a function of 2θ ranging between 5-60°.

3.6.4 Scanning Electron Microscopy (SEM)

All particulate and film-based samples particles were examined using a Hitachi S-4800 scanning electron microscope (SEM) at an accelerating voltage of 0.7 kV. The samples were mounted on adhesive carbon black and sputter coated with a gold/palladium layer.

3.6.5 Atomic Force Microscopy (AFM)

The surface topographies of the PLA and PLLA microparticles and the PLA and PLLA substrate films before and after surface grafting and before and after surface degradation were determined using the AFM tapping mode (Nanoscope IIIa multimode, Digital Instruments, Santa Barbara, CA). A silicon tip (NSC14/no Al, Mikromasch, Estonia) with a normal spring constant (k) of 5.7 N/m and a resonant frequency (f₀) of 110-220 kHz was used together with an EV scanner in the tapping mode. The same instrument was equipped with a picoforce extension (Veeco Instruments, Santa Barbara, CA) to measure force interactions between the different combinations of colloidal probes and substrate films. The force measurements were conducted in liquid or aqueous media. AFM Tipless cantilevers, 250 µm in length and 35 µm in width and with a normal spring constant in the range of 0.35-1.2 N/m (NSC12/no Al, Mikromasch, Estonia), were initially calibrated to determine the correct spring constant values using AFM Tune IT v2.5 software (ForceIT, Sweden), which is based on a thermal noise method. After the calibration, a spherical PLA particle was glued to the free-end of the tipless cantilever with the aid of a 3D manual micromanipulator and a reflecting light microscope. Before gluing the colloidal probes, all tipless cantilevers were cleaned by exposure to UV-plasma treatment for approximately 2 min at medium power (Harrick Plasma, Inc., USA). The AFM liquid cell and all other parts related to the liquid cell were cleaned by soaking in Hellmanex II (Helma GmbH & Co., Germany) for at least 1 h, followed by continuous rinsing with Milli-Q water and finally, drying in a flow of filtered nitrogen gas. The colloidal probe in the liquid cell was inserted into the AFM head for the force measurements. The probe was then brought into close proximity to the
substrate surface. The lowest concentration of NaCl, 0.1 mM, was then injected into the liquid cell using a 10-mL syringe, and the system was allowed to equilibrate for approximately 10 min. The approach rate of the colloidal probe toward the substrate surface was 250 nm/s. Force measurements were also performed in two other salt solutions, 1 mM and 10 mM. The force profiles, obtained from the AFM measurements, were normalized and evaluated with the DLVO and AdG models (Eqs. 3 and 4).

3.6.6 Zeta potential
The zeta potentials of the polyelectrolyte multilayers attached to the grafted microspheres were determined using a Nanozetasizer with a He-Ne laser source at 633 nm (Zetasizer ZEN 3600, Malvern Instruments, and U.K). The microspheres were injected into the zeta potential cell and allowed to equilibrate for 2 min. During the measurement, the Slomuchowski method\textsuperscript{83} was used to obtain the zeta potential.

3.6.7 Compression testing
Mechanical testing was carried out on an Instron 5566 instrument (Instron, UK) with a load cell of 10 kN and a compressive rate of 10 % thickness/min. The 3D-microspheres scaffolds had a height ranging between 5-8 mm and a diameter range of 8-10 mm. The specimens were first preconditioned under 50% humidity, 23°C for 48 h. The stress and strain values at break points and the Young’s moduli were recorded for comparison.

3.6.8 Particle Size Distribution (PSD)
The particle size distributions of the microparticulate PLLA samples before and after PEMs buildup were determined using a laser diffraction spectrometer with a He-Ne laser at 632.8 nm (Model HELOS/KR-H3522, Sympatec GmbH, Clausthal-Zellerfeld, Germany) at a distance of 10 mm to the spray outlet according to ISO 13320:2009. The particles had previously been dispersed using a RODOS dispersing unit under a reduced pressure of 2 bar. The HELOS was run with a focal distance of \( f_{R3} = 100 \) mm to cover between 0.5 μm and 175 μm. The measurement was performed for 1.5 s for each sample. The particle size distribution (PSD) was later evaluated using a Fraunhofer diffraction model.

3.6.9 Porosity measurement
The porosity of the 3D scaffolds was measured by a \( \mu \)CT device (SkyScan 1172 Scanner, Belgium) at 40 keV and 2.4 μm voxel. Samples were randomly selected and characterized by CTan (CT Analyser, ver. 1.5.0, SkyScan) and CTvol (CT Vol Realistic, ver. 1.9.4, SkyScan) software. Three-dimensional models were later
reconstructed by NRecon (ver.1.6.9, SkyScan) and CTan (v.1.14.4, SkyScan) software.

3.6.10 Conductivity measurement

The 3D scaffolds were initially doped with 1 mol/L HCl for 24 h. Undoped 3D scaffolds were also prepared as references. After Cl\(^-\) doping, the scaffolds were dried in an evacuated oven for 48 h so that the conductivity was not affected by water content. Microsphere pellets, with diameters in the range of 0.6-0.9 cm and thickness ranging from 0.3 to 0.4 mm, were made by compression molding using a pressure of 150 kN/m\(^2\) at 100°C for 10 min. The voltages of the undoped and 1 mol/L HCl-doped 3D microsphere scaffolds were measured via a 1 mA pulsed electric current. The 3D materials were later positioned between two symmetrical cylindrical stainless steel plates, and the electrical conductivity was evaluated using Pouillet’s law.\(^{84}\)

3.6.11 pH measurement

The pH of the surface degradation media was evaluated using a pre-calibrated pH-meter equipped with an Ag/AgCl electrode (Hamilton, USA).
4 RESULTS AND DISCUSSION

The development of a non-destructive ‘grafting-from’ technique using micro and macrosized particles opens new means of investigating the behavior of polymer grafts covalently attached to PLA particle surfaces. Steric stabilization induced by hydrophilic grafts covalently attached to PLA particles demonstrate that the particles could be used to overcome particle agglomeration due to the presence of long-range repulsive forces of the grafted polymeric particles in aqueous solutions. A 3D microsphere scaffold suitable for bone tissue regeneration was successfully created using a combination of the developed surface grafting technique and the LbL method. Additionally, the mechanical properties of functionalized, 3D microsphere scaffolds were similar to the compressive stiffness and porosity of human cancellous bone. Furthermore, surface grafting on different geometries, i.e., PLLA films and microparticles, was shown to control the surface properties in terms of morphology, chemistry and topography during surface degradation.

4.1 THE FABRICATION OF PARTICLES

To fabricate the different geometries of the particulate materials, three well-known techniques, O/W emulsion, spray drying and cryogenic milling, were used. The first two methods produced spherical particles, while the third technique yielded particles with irregular shapes. The number average molecular weight (Mn) as characterized by SEC demonstrated that no significant differences were observed between the particles produced by the O/W and milling techniques. However, the particles fabricated by these techniques had a slightly lower Mn than that of the PLA pellet (Table 1). The milling technique resulted in a higher particle yield and hence, was more efficient. These irregular particles were later used to develop a surface modification method via the ‘grafting-from’ technique. Changes in surface chemistry, morphology, and topography were also observed using the spherical particles produced by the O/W emulsion technique. In contrast to the particles made by the O/W emulsion technique,
no changes in $M_n$ were observed between the synthesized PLLA and the particles produced by the spray drying technique (Table 1). Additionally, the spray drying technique produced higher yields and smaller particles than the O/W emulsion technique.

**Table 1.** The number average molecular weight ($M_n$) and $\bar{D}$ of PLA and PLLA before and after particle fabrication

<table>
<thead>
<tr>
<th>Sample</th>
<th>$M_n^a$</th>
<th>$\bar{D}^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLA pellet</td>
<td>166,000±3,800</td>
<td>1.46</td>
</tr>
<tr>
<td>cryogenic milled PLA particles</td>
<td>145,000±200</td>
<td>1.62±0.01</td>
</tr>
<tr>
<td>emulsified PLA particles</td>
<td>146,000±10,300</td>
<td>1.62±0.05</td>
</tr>
<tr>
<td>Synthesized PLLA</td>
<td>127,000±900</td>
<td>1.20</td>
</tr>
<tr>
<td>Spray dried PLLA particles</td>
<td>123,000±700</td>
<td>1.20</td>
</tr>
</tbody>
</table>

$a$Measured by SEC (polystyrene standards for calibration and chloroform as an eluent).

### 4.2 SURFACE MODIFICATION THROUGH THE ‘GRAFTING-FROM’ TECHNIQUE

A surface modification technique via a ‘grafting-from’ method was developed using three unsaturated hydrophilic monomers, AAm, AA, and MAH, and two different particle geometries, irregular and spherical microparticles. The $M_n$ of the particles were monitored to verify that degradation did not occur during the surface grafting. Along with $M_n$, changes in the surface chemistry, morphology and topography of the particles before, during, and after surface grafting were characterized to confirm that the grafted chains were covalently attached to the particles and that the method itself was applicable to all types of polymeric particles.
4.2.1 Non-destructive character of ‘grafting-from’ method

During UV-irradiation, BP absorbs photon energy and abstracts hydrogen atoms from the substrate, leading to the formation of radicals on the substrate surface. The surface radicals then undergo reactions with hydrophilic monomers, and polymerization proceeds. To demonstrate the non-destructive nature of the ‘grafting-from’ method, the $M_n$ of the PLA particles after surface activation and surface grafting were determined (Table 2).

**Table 2.** The number average molecular weights ($M_n$) and $D$ of PLA particles during activation and after surface grafting.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$M_n$ $^b$</th>
<th>$D$ $^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface-activated irregular particles $^a$</td>
<td>144,000±3,300</td>
<td>1.64±0.02</td>
</tr>
<tr>
<td>Surface-activated spherical particles $^a$</td>
<td>142,000±13,000</td>
<td>1.66±0.17</td>
</tr>
<tr>
<td>Surface-activated irregular particles and UV-irradiated for 1.5 h</td>
<td>158,000±3,600</td>
<td>1.54±0.02</td>
</tr>
<tr>
<td>Surface-activated spherical particles and UV-irradiated for 1.5 h</td>
<td>131,000±5,400</td>
<td>1.72±0.14</td>
</tr>
<tr>
<td>PLA-g-PMAH of irregular particles for 1.5 h</td>
<td>126,000±3,600</td>
<td>1.46±0.02</td>
</tr>
<tr>
<td>PLA-g-PMAH of spherical particles for 1.5 h $^c$</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

$^a$The PLA particles were surface-activated in ethanol for 20 min. $^b$Measured by SEC. $^c$Insoluble in chloroform.

The surface activation induced by UV-irradiation showed no significant effect on the $M_n$. Unfortunately, the $M_n$ of the particles after surface grafting could not be determined except for PMAH-grafted irregular particles due to the very limited solubility of the polymer grafts in the SEC analyses. Graft and copolymers typically show changes in their molecular weights as a result of the different hydrodynamic volumes of the polymers in the organic solvent used during SEC measurement. Depending on the thickness of the grafting layer, the molecular weight of the polymer grafts was determined by SEC. $^7,18$ Unfortunately, the substrates and their grafting layers could not be measured due to a thick or dense grafting layer.
4.2.2 Evaluation of new functionalities on the PLA backbone

The composition of the particle surfaces were evaluated by FTIR to confirm the success of the ‘grafting-from’ method.

![ATR-FTIR spectra of irregular PLA particles before and after surface grafting with AAm monomer for different periods of time.](image)

**Figure 1.** ATR-FTIR spectra of irregular PLA particles before and after surface grafting with AAm monomer for different periods of time.

The neat PLA particles displayed the characteristic band at 1745 cm\(^{-1}\) corresponding to the ester C=O bond (Figure 1). After 30 min of AAm grafting, a shoulder was formed at 1653 cm\(^{-1}\), corresponding to the stretching vibrations caused by an amide I bond. The primary amide (amide II bond) was detected at 1615 cm\(^{-1}\) after 60 min of surface grafting\(^{7,18,85}\) this confirmed the existence of the AAm grafting layer on the PLA surface. The absorption band of the C-N stretching bond is typically detected at 1440-1200 cm\(^{-1}\) but were unfortunately difficult to identify due to overlapping bands from the PLA substrate functional groups.
For PLA-g-PAA, a shoulder was detected at 1747 cm\(^{-1}\), which corresponds to the absorption band of the carboxylic acid of PAA.\(^{86}\) This shoulder was significantly wider after exposure to over 60 min UV-irradiation (Figure 2). For PLA-g-PMAH, the extent of the grafting observed was low, and the stretching bond of the anhydride could not be captured in the 1840-1720 cm\(^{-1}\) region.\(^{86}\) The C=O stretching absorption peak was slightly observed, which indicates a low amount of PMAH attached to the PLA particle surface (Figure 3). A monolayer of MAH is commonly created on the substrate surface,\(^7\) which is difficult to be observed through FTIR analysis due to the large penetration depth of FTIR of approximately 1 µm.

**Figure 2.** ATR-FTIR spectra of irregular PLA particles before and after surface grafting with AA monomer for different periods of time.
Figure 3. ATR-FTIR spectra of irregular PLA particles before and after surface grafting with MAH monomer for different periods of time.

AAm and MAH monomers were surface-grafted on the spherically shaped particles to evaluate the versatility of the ‘grafting-from’ technique for other geometries. After 90 min of grafting, the surface chemistries of the grafted microspheres were analyzed (Figure 4). The FTIR spectra were similar to those of the grafted irregular particles. Amide bands were observed for the spherically shaped particles and the irregularly shaped particles; the anhydride peaks at 1840-1720 cm\(^{-1}\) of PMAH when grafted onto microsphere surfaces could not be observed.
4.2.3 Morphology and topography of grafted microspheres

The morphology of the PLA microspheres before and after surface grafting were analyzed by SEM to determine the influence of the surface grafting technique on the morphology of the substrate surfaces. Before grafting, the SEM images revealed random holes over the microsphere surfaces, likely caused by the rapid evaporation of solvents and humidity during particle fabrication (Figure 5). An average particle diameter of $36 \pm 13 \mu m$ was determined. Because many holes were present at the surface, the neat PLA microspheres were used as a morphological baseline to represent complete surface coverage. A few holes were also detected after irradiating the samples in ethanol for 90 min in the absence of monomers. Ethanol did not induce any visible changes on the morphology of the PLA microspheres. Furthermore, no holes were detected when acrylamide was used as a hydrophilic monomer to surface graft the PLA microspheres, indicating complete surface coverage; an average microsphere diameter of $63 \pm 28 \mu m$ was determined. For the PLA-g-PMAH, the morphology on the surface was similar to the neat PLA microspheres, again indicating a low degree of grafting, as previously indicated by the FTIR spectra.
Results and Discussion

Figure 5. The morphology of surfaces of (A) the neat PLA particles, (B) the PLA particles irradiated for 90 min in ethanol, (C) PLA-g-PAAm, and (D) PLA-g-PMAH grafted for 90 min, respectively.

In addition to the surface morphology, the surface topography was used to evaluate the texture of the surface grafted particles when compared with their reference (Figure 6). The neat PLA sphere had a smooth texture with an rms roughness of 5.33 nm, while the PLA sphere under UV-irradiation in ethanol had a relatively rough texture with an rms roughness of 37.2 nm. The different monomers that were surface grafted on the PLA spherical particles induced different surface topographies. The PAAm grafting layer had a smoother texture (rms roughness = 16.9 nm) when compared with the PMAH grafting layer (rms roughness = 42.8 nm).
Results and Discussion

Figure 6. The topography of the surfaces of (A) the neat PLA microspheres, (B) PLA microspheres irradiated for 90 min in ethanol, (C) PLA-g-PAAm microspheres, and (D) PLA-g-PMAH microspheres grafted for 90 min.

The results again indicate that the PAAm chains resulted in a better surface coverage than the PMAH chains as determined by the FTIR and SEM analyses. The phase image from AFM indicated that the monomer-carrying solvent significantly affected the rms roughness, despite no significant change in $M_n$ before and after surface treatments.

4.3 THE STABILITY OF GRAFTED SPHERICAL PARTICLES

Polymer grafts attached to the surfaces of microspheres can be used to prevent agglomeration in aqueous solutions. Thus, the interacting forces of the two hydrophilic polymer grafts, PAAm and PAA, were analyzed using colloidal probe AFM. The former polymer is a neutral polymer, while the latter is sensitive to pH changes. The microspheres and film substrates were surface-grafted for 90 min using the same procedure previously described. Again, the topographies of the dry and wet polymeric substrates were monitored. Afterward, the force interactions of two different systems, asymmetric (only one substrate was surface-grafted) and
symmetric (both substrates were surface-grafted or not surface-grafted), were measured in different concentrations of salt solutions from 0.1 to 10 mM in MilliQ water.

### 4.3.1 Topographies of different PLA substrates in air and in solutions

The surface topography of the neat and grafted PLA microspheres and films were analyzed by AFM. The neat PLA microspheres had smooth surfaces (Rq = 3.3 ± 1.6 nm) (Table 3; Figure 7A). The PLA films fabricated by solvent casting had similar smooth surface topographies as the PLA microspheres (Rq = 4.0 ± 1.0 nm) in air. The AFM height image in air revealed a 19.8 ± 2.1 nm rms surface roughness of the PLA-g-PAAm microspheres, whereas a rougher 34.0 ± 1.6 nm rms surface roughness topography was found for the PLA-g-PAA microspheres.

**Table 3.** Surface roughness (Rq) of PLA, PLA-g-PAAm, and PLA-g-PAA microspheres and films in air and salt solutions.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Surface roughness (Rq) (nm)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PLA sphere</td>
<td>PLA film substrate</td>
</tr>
<tr>
<td></td>
<td>Air</td>
<td>NaCl solution</td>
</tr>
<tr>
<td>PLA</td>
<td>3.3±1.6</td>
<td>4.0±1.0</td>
</tr>
<tr>
<td>PLA-g-PAAm</td>
<td>19.8±2.1</td>
<td>18.0±1.0</td>
</tr>
<tr>
<td>PLA-g-PAA</td>
<td>34.0±1.6</td>
<td>39.0±5.0</td>
</tr>
<tr>
<td>PLA-g-PAA (pH=7.4)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Figure 7. The topography of the surfaces of (A and E) neat PLA, (B and F) PLA-g-PAAm, (C and G) PLA-g-PAA, and (D and H) PLA-g-PMAH films in air. The height and phase are shown by top and bottom images, respectively. All phase images of AFM were scanned over 5 x 5 µm.

Based on the surface roughness calculations, these PLA film substrates could be used as the substrates in the force measurements instead of the PLA-based microspheres because no significant differences in the surface roughness were observed for the neat PLA, PLA-g-PAAm and PLA-g-PAA particles and films in air (Table 3). Water beads were observed on the surfaces of the neat PLA films when they were immersed in 0.1 mM salt solutions at pH 5.6 (Figures 8A and 8D); no self-organization of water beads on the surfaces of neat PLA in air were observed (Figures 7A and 7E). Water rearrangement occurring on the surfaces of hydrophobic polymers is believed to induce such self-rearranging water beads. No surface roughness alterations were observed for the neat PLA films in air or in the different salt solutions (Table 3).
In contrast to what was observed on the hydrophobic surfaces, water beading did not occur on the hydrophilic polymers grafted on the PLA surface at pH 5.6 (Figures 8B and 8C). The phase images from AFM indicated changes in the conformations of the PAAm polymer chains in salt solutions (Figure 8E), thus resulting in a rougher surface than for PAAm polymer chains in air. In contrast to PLA-g-PAAm, the PLA-g-PAA substrates did not demonstrate any measurable changes in conformation at the two lower salt solution concentrations at pH 5.6 (Figures 8C and 8F) because the surface roughnesses of the grafted chains in the wet state were approximately similar to their rms values in air. At a high pH, i.e., 7.4, the chain conformation of the PAA grafts changed at the highest salt concentration, 10 mM. A conformational change was observed in the PLA-g-PAA grafts, which were rougher than those of the same surface of interest at a higher pH.

4.3.2 Force interactions of PLA substrates of different systems

The force interactions of neat PLA substrates and the grafted PLA substrates, i.e., PLA-g-PAAm and PLA-g-PAA, respectively, were measured for the different combinations given below (Table 4). The force interactions were performed in different salt concentrations at pH 5.6 unless otherwise stated.
Table 4. A set-up of force measurement performed in salt solutions, type of interaction and result of force measurement of two interacting substrates.

<table>
<thead>
<tr>
<th>System</th>
<th>Colloidal probe</th>
<th>Film substrate</th>
<th>Type of interaction</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PLA</td>
<td>PLA</td>
<td>hydrophobic/hydrophobic</td>
<td>Attraction</td>
</tr>
<tr>
<td>2</td>
<td>PLA-g-PAAm</td>
<td>PLA-g-PAAm</td>
<td>hydrophilic/hydrophilic</td>
<td>Repulsion</td>
</tr>
<tr>
<td>3</td>
<td>PLA-g-PAA</td>
<td>PLA-g-PAA</td>
<td>hydrophilic/hydrophilic</td>
<td>Repulsion</td>
</tr>
<tr>
<td>4</td>
<td>PLA-g-PAA</td>
<td>PLA-g-PAAm</td>
<td>hydrophilic/hydrophilic</td>
<td>Repulsion</td>
</tr>
<tr>
<td>5</td>
<td>PLA</td>
<td>PLA-g-PAAm</td>
<td>hydrophobic/hydrophilic</td>
<td>Repulsion</td>
</tr>
<tr>
<td>6</td>
<td>PLA</td>
<td>PLA-g-PAA</td>
<td>hydrophobic/hydrophilic</td>
<td>Attraction</td>
</tr>
</tbody>
</table>

A short-range repulsion of the symmetric hydrophobic system, i.e., the PLA/PLA system was detected at a salt concentration of 0.1 mM before observing a jump-into contact of the colloidal probe (Figure 9A). For this system, at this lowest salt concentration, the repulsive interactions were in agreement with DLVO theory. A surface potential of PLA ($\varphi_{PLA}$) of approximately -12 mV was measured (Figure 9C). This type of repulsion may have occurred due to the adsorption of negatively charged ions (Cl$^-$), a phenomenon commonly known as ion-specific adsorption, on the surface of hydrophobic polymers and leads to a weakly repulsive double layer effect. The repulsive interaction is then reduced when the screening effect increases with increasing salt concentration. At a separation distance of 20 nm, attractive interactions were detected when immersing a hydrophobic system at the two higher salt concentrations, 1 mM and 10 mM (Figures 9B and 9C). In general, an attraction is seen when two hydrophobic surfaces interact at longer distances ($D \geq 15$-$20$ nm). Hence, the hydrophobic interaction failed to prevent agglomeration when dispersing colloidal PLA particles in suspension due to the presence of attractive forces. A better system should be designed to prevent particle agglomeration.

As in the symmetric hydrophobic system, attractions were recorded on the approach and retraction curves of one of the asymmetric systems, PLA/PLA-g-PAA (Table 4) in salt concentrations ranging from 0.1 to 10 mM. Attractive forces were also detected at long separation distances of 30 to 60 nm. Adhesive forces were detected on the retraction curves, as indicated by 1.3-2.7 mN/m pull-off forces.
Figure 9. The profiles of normalized forces detected when a PLA sphere and PLA film were brought into contact. (A) Five distinct locations on the substrate surface showed approach (green)-retraction (blue) curves in 0.1 mM salt solution, (B) typical approach force curves observed from different salt concentrations, 0.1 mM (green), 1 mM (red), and 10 mM (blue), (C) the solid, dashed, and dotted grey lines refer to the calculated DLVO force in different salt concentrations, namely 0.1, 1, and 10 mM.

All symmetric hydrophilic systems (Figures 10-11) and the asymmetric PLA/PLA-g-PAAm system (Table 4) showed long-range repulsions for the approach and retraction curves. Additionally, the profiles of the normalized forces of these systems were not affected by the different salt concentrations. The DLVO theory, where the EDL exists, did not fit well with the force curves of these symmetric systems and the asymmetric PLA/PLA-g-PAAm system at pH 5.6. However, an exception was observed for one of the force profiles of the PLA-g-PAA/PLA-g-PAA system, which agreed well with DLVO theory at the highest salt concentration at pH 7.4 (Figure 11). The presence of steric force could be expressed using the AdG model, which predicts
grafted polymer brushes stretching away from the surface and no chain interpenetration. Hence, the AdG model could be used to represent steric repulsion through the measured force profiles (Figures 10-11). Additionally, the presence of steric repulsion can later overcome the formation of aggregated colloidal particles in suspension; these designed systems are well-dispersed in aqueous solutions. Long-range repulsive interactions were observed for PLA-g-PAAm/PLA-g-PAAm (Figure 10). Based on a force curve fitting to the AdG model, a 250 nm grafting layer thickness ($L_o$) and a grafting density ($\Gamma$) and an average grafting site ($s$) were approximated to be $0.14 \pm 0.07$ chains/nm$^2$ and $2.91 \pm 0.8$ nm, respectively (Figure 10B).

**Figure 10.** The profiles of the normalized forces detected when a PLA-g-PAAm sphere and PLA-g-PAAm film were brought into contact. (A) Five distinct locations on the substrate surface showed approach (green)-retraction (blue) curves in 0.1 mM of salt solution, (B) typical approach curves observed from different salt concentrations, 0.1 mM (green), 1 mM (red), and 10 mM (blue). The solid, dashed, and dotted grey lines refer to the DLVO calculated force in different salt concentrations, namely 0.1, 1 and 10 mM. The solid black line shows the AdG fitting curve in 0.1 mM salt solution.

As previously stated, the approach and retraction curves of the symmetric PLA-g-PAA/PLA-g-PAA hydrophilic system exhibited purely repulsive forces at pH 5.6 (Figure 11). This is in contrast to the other PLA-g-PAAm/PLA-g-PAAm symmetric system, where the repulsive forces of PLA-g-PAA/PLA-g-PAA system varied from one point to another on the substrate surface (Figure 11A). Similar to PLA-g-PAAm/PLA-g-PAAm, the experimental data of the force curves agreed well with the AdG model, resulting in an $L_o$ of 139 nm, a $\Gamma$ of $0.06 \pm 0.03$ chains/nm$^2$ and an $s$ of $3.74 \pm 1.65$ nm (Figure 11B). The PAA grafts exhibited a conformational transition when the pH increased to 7.4, as shown by their altered surface roughness. The contribution of steric force dominated this symmetric hydrophilic system at two lower salt concentrations, 0.1 and 1 mM, while the DLVO theory had a good
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At the highest salt concentration, 10 mM, the system showed a mathematical fit leading to a surface potential of \( \varphi_{PA} = 45 \) mV for PLA-g-PAA/PLA-g-PAA. This highlights a contribution of electrostatic forces working between the symmetric hydrophilic systems of PLA-g-PAA at the highest pH. The steric forces dominated for two lower salt concentrations, 0.1 mM and 1 mM, as indicated by the AdG model, which showed a good fit to the force profiles and \( L_0 \) values of 149 and 107 nm were determined, respectively (Figure 11C). However, the \( L_0 \) for the system was reduced when the salt concentration was increased from 0.1 to 1 mM at pH 7.4.

Figure 11. The profiles of normalized forces detected when a PLA-g-PAA sphere and PLA-g-PAA film were brought into contact. (A) Five distinct locations of approach (green)-retraction curves (blue) performed in 0.1 mM of salt solution, (B) the approach curves observed at different salt concentrations, 0.1 mM (green), 1 mM (red), and 10 mM (blue). The solid, dashed, and dotted grey lines refer to the DLVO calculated force of different salt concentrations, namely 0.1 mM, 1 mM, and 10 mM. The solid black line shows the AdG fitting curve of 0.1 mM of salt solution at pH 5.6. (C) The approach curves observed at different salt concentrations, 0.1 mM (green), 1 mM (red), and 10 mM (blue) at pH 7.4. The solid, dashed, and dotted grey lines refer to the DLVO calculated force of different salt concentrations, namely 0.1 mM, 1 mM, and 10 mM. The black solid line shows the AdG fitting curves of 0.1 mM and 1 mM of salt solutions at pH 7.4.
Additionally, the $L_o$ decreased to approximately 109 nm when the two hydrophilic substrates with different polymer grafts, PAAm and PAA, interacted; however, a long-range repulsion still dominated. The approach and retraction curves of this mixed symmetric system, PLA-g-PAA/PLA-g-PAAm, were not quite the same due to plastic deformation when the soft polymer grafts were indented during force measurement.

The force interaction naturally occurring between weak polyacids constitutes a complex phenomenon dependent on several parameters, including the pH of the solution, the solvent, the salt concentration, and the grafting layer density.\textsuperscript{91,92} The conformational chain transitions of the polymer grafts from flattened or mushroom shapes or to an extended brush could then be observed by altering these factors. Different force interactions of the weak polyacid, PAA, have been shown to rely on ionic strength and pH. The PAA grafts seemed partially or not charged at pH 5.6 likely because no electrostatic interactions were observed for the different salt concentrations, leading to similar force interactions caused by the steric forces (Figure 11B). Additionally, no changes were observed in surface roughness at the two lower salt concentrations, indicating that the conformational chain transition was not dependent on these two lower salt concentrations (Table 3). When the pH of the aqueous solution was increased to 7.4 (Figure 11C), the symmetric system changed from being dominated by steric to electrosteric forces as indicated by the presence of electrostatic interactions at the highest salt concentration. At 0.1 mM salt solution, steric repulsion caused a chain extension for all different salt concentrations; no electrostatic contribution was observed. However, a purely electrostatic repulsion contributed to the PAA symmetric hydrophilic system at the highest salt concentration, 10 mM, and may have induced the conformational chain transition of the polymer grafts.

Because the long-range repulsions were dominated by steric or electrosteric forces in the aqueous systems, all symmetric hydrophilic systems designed here could be used for applications where the prevention of colloidal particle agglomeration is required.

4.4 FABRICATION OF 3D SCAFFOLDS

To design advanced materials for bone tissue engineering, surface grafting was combined with the LbL approach to alternately adsorb PEMs onto PAA-surface grafted microspheres. Two different weak polyelectrolytes, PAA and PAH, and a neutral polymer, PAAm, were used to evaluate the assembly of PEMs onto the grafted and non-grafted PLLA particles. A series of different systems were prepared.
to demonstrate the growth of these weak polyelectrolytes at two different pH values (Table 1, Chapter 3).

Figure 12. Three-dimensional microsphere scaffolds in comparison to 10-cent Euro coins. The scaffolds were fabricated from PAA-grafted PLLA microspheres alternately assembled with (left) (PAA/PAH)$_6$ multilayers or PAH$_{2.5}$, (middle) (PAA/PAH/GO)$_6$ multilayers or PAH$_g$GO, and (right) (PAA/PAH/Fe$_3$O$_4$)$_6$ multilayers or PAH$_g$FeO. All scaffolds were assembled at pH 2.5.

The PEM-coated microparticles were packed into cylindrically shaped templates to produce 3D scaffolds (Figure 12, left). The grafted PLLA microspheres onto which PEMs were physically adsorbed remained intact when exposed to high-shear mixing and ultrasonication at high amplitude in aqueous solutions. The non-grafted PLLA microspheres could not be molded into a permanent 3D shape when the PEMs were not assembled on the surfaces, and they easily collapsed after the 3D cylindrical template was removed. Different functional nanosized species, i.e., GO (Figure 12, middle) and Fe$_3$O$_4$ (Figure 12, right) were also alternately adsorbed onto the particles and shaped into 3D structures. The ability to functionalize nanomaterials would be applicable to a broad spectrum of uses in bioelectronics, optoelectronics, and the pharmaceutical and medical fields. Because graphene is one of the strongest materials with a Young’s modulus of approximately 1 TPa,$^{93}$ and because it has been
demonstrated to accelerate the cells differentiation,\textsuperscript{94,95} graphene and its derivatives, including GO, have recently been exploited to increase the mechanical performance of scaffolds for tissue engineering.\textsuperscript{96,97} Enhanced cell differentiation has also been demonstrated for Fe$_3$O$_4$ nanoparticles.\textsuperscript{98,99} The functionalization of GO nanomaterials onto 3D microsphere scaffolds assembled via LbL approach resulted in a grey-colored scaffold (Figure 12, middle), darker than the Fe$_3$O$_4$-functionalized 3D scaffold (Figure 12, right).

4.4.1 Zeta potential during the assembly of PEMs

The measurement of the zeta potential plays a crucial role in monitoring the charge reversal assembly of polyelectrolytes on microsphere surfaces.\textsuperscript{100} The first layer of the PLLA-g-PAA microspheres had a zeta potential of -15 ± 2 mV at pH 2.5 (Figure 13); the zeta potential became more negative, -30 ± 1 mV, as the pH of solution increased due to the formation of ionized acrylic acid groups (Figure 13). The zeta potential sign reversed as the cationic polyelectrolyte PAH was adsorbed, to 23 ± 2 mV and 24 mV at pH 2.5 and 5, respectively, as a consequence of charge overcompensation. However, the adsorption of the neutral PAAm did not induce a large shift in the zeta potential, only -3.3 mV ± 0.2, due to the neutral character of the PAAm chains (Figure 13). The zeta potential again reversed to a negative value with the adsorption of the third layer of PAA. The sign of the zeta potential of the outermost layer of the PAA/PAAm multilayers was less negative than for the PAA/PAH multilayers at pH 2.5. A more negative zeta potential value was observed for PAA/PAH multilayers at pH 5. The alternating adsorptions of neutral polymers and cationic polyelectrolytes continually reversed the zeta potential sign. A zeta potential reversal was observed with each subsequent adsorbed polyelectrolyte layer,\textsuperscript{101} indicating the deposition of PEMs on grafted microsphere surfaces.
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Figure 13. Zeta potential (ξ) as a function of number of PEM layers for PAMg (dotted line), PAH2.5g (grey), and PAH5g (black). The positive and negative zeta potentials correspond to PAH and PAA layers, respectively. Zeta potentials of PAAm layer are less negative than PAA layer.

4.4.2 Evaluation of new functional groups on the microsphere surfaces

The new functionalities attached to the PAA-grafted PLLA microsphere surfaces after the assembly of PEMs were characterized using FTIR and later compared with those of their non-grafted analogues (Figure 14). The surface chemistry of the microparticulate shows the highly dense multilayer films as indicated by the two main wavenumber (ʋ) regions, i.e., ʋ: 1800-1500 cm⁻¹ and ʋ: 3800-2300 cm⁻¹.

As expected, neat PLLA microspheres showed an absorption peak of ester groups at 1747 cm⁻¹ (Figures 14A and 14C). In all non-grafted microspheres, the sequential adsorption of PAA/PAAm or PAA/PAH multilayers onto the microsphere surfaces was indicated by the distinct absorption bands different from the first, third and sixth bilayers. Here, the hydrophobic character of PLLA was shown to be reactive to the adsorption of weak polyelectrolytes physically driven via the electrostatic (or hydrogen bonding) interactions during the assembly process.
Figure 14. ATR-FTIR spectra of neat PLLA microspheres (black) and alternating polyelectrolyte multilayers (PEMs) adsorbed onto the surface of (A) non-grafted PLLA microspheres with the following systems: PAMₐ (wine); PAH₂.₅ₐ (dark cyan); PAH₅ₐ (light blue) at 1800-1500 cm⁻¹ and (B) at 3800-2300 cm⁻¹ (C) PAA-grafted PLLA microspheres with the following systems: PAMₐ (red); PAMₐGO (magenta); PAMₐFeO (orange); PAH₂.₅ₐ (green); PAH₅ₐGO (violet); PAH₅ₐFeO (gray); PAH₅ₐ (blue) in the region of 1800-1500 cm⁻¹ and (D) at 3800-2300 cm⁻¹ (E) at 1100-600 cm⁻¹ (F) XRD pattern of particulate samples with or without GO functionalization.
The first bilayer of the PAA/PAAm multilayers on the non-grafted PLLA microspheres was not clearly detected; however, a tiny absorption band appeared near 1623 cm\(^{-1}\), indicative of the primary amide. Additionally, the IR patterns of the PAA/PAH multilayers assembled on the surfaces at pH 2.5 and 5 were also detected (Figure 14A). The absorption bands at 1550-1540 cm\(^{-1}\) of the C=O groups originating from the PAA chains agreed well with the asymmetric stretching bond of the ionized carboxylate (COO\(^{-}\)) groups and the absorption band at 1725 cm\(^{-1}\), which represents the characteristic C=O bond of the carboxylic acid (COOH) groups.\(^{102}\) After the deposition of the third bilayer, higher intensity COO\(^{-}\) absorption bands of (PAA/PAH)\(_6\) multilayers at 1600-1500 cm\(^{-1}\) assembled at pH 2.5 were observed. These increased intensities may be due to the hydrophobic character of the base substrate and the effect of the added polycation on the ionization of carboxylic functional groups in weak polyacids and bases.\(^{102}\) The N-H and C-H stretching absorption bands of the PAH layers in the 3800-2300 cm\(^{-1}\) region increased when the pH solution increased (Figure 14B). However, no strong overlapping N-H and C-H stretching bands were recorded.

The growth of the PEMs on the PAA-grafted PLLA surfaces was also observed from the first to sixth bilayer (Figure 14C). In contrast to the non-grafted samples, the surface grafting strongly affected the peak area in the 1800-1500 cm\(^{-1}\) region where the C=O stretching band of PLLA overlapped with those of the carboxylic acid (COOH) and ionized carboxylate (COO\(^{-}\)) groups of the PAA chains and the amide I and II bands of the PAAm chains (Figure 14C). The N-H and C-H stretching bands in the 3800-2300 cm\(^{-1}\) region also increased after the sequential adsorption of PEMs (Figure 14D). PAM\(_g\) demonstrated a larger peak area of 20 Å/cm than the PAM\(_n\) peak area, 17 Å/cm, at 1800-1500 cm\(^{-1}\). The N-H and C-H stretching peaks of PAM\(_g\) also had a larger peak area than that of PAM\(_n\) at 15 Å/cm and 8 Å/cm, respectively. In contrast to the peak area of (PAA/PAAm)\(_6\) multilayers, the peak areas of PAH2.5\(_g\) and PAH2.5\(_n\) from 1800-1500 cm\(^{-1}\) were similar at 20 Å/cm. However, the N-H and C-H stretching bands of PAH2.5\(_g\) exhibited a larger peak area than PAH2.5\(_n\) (Table 5). This indicated that the (PAA/PAH)\(_6\) multilayers were more dense on the grafted surfaces than on the non-grafted surfaces. The physically adsorbed (PAA/PAH)\(_6\) multilayers on the non-grafted and grafted surfaces at pH 5 had lower intensity COOH and COO\(^{-}\) absorption bands than those of the same multilayers deposited at the lower 2.5 pH. The alternating assembly of the PEMs on the surfaces of both non-grafted and PAA-grafted PLLA microspheres is a complex process that affects the conformation of the acrylic acid groups of PAA and the amine-methylene groups of PAH during the adsorption of PEMs at different pH.

The functionalization of the GO and Fe\(_3\)O\(_4\) nanomaterials on the (PAA/PAH)\(_6\) multilayers at pH 2.5 showed no increases in absorption bands at 1800-1500 cm\(^{-1}\).
Because the FTIR spectra of these nanomaterials completely overlapped, they had the same peak area of 19 A/cm (Table 5). These nanomaterials decreased the intensities of the amine-methylene absorption bands of the PAA/PAH multilayers. However, the Fe\textsubscript{3}O\textsubscript{4} functionalization had a larger area at 3800-2300 cm\textsuperscript{-1} than the GO functionalization (Table 5). When these nanomaterials were functionalized on the (PAA/PAA\textsubscript{m})\textsubscript{6} multilayers, their peak area in the 1800-1500 cm\textsuperscript{-1} region varied when compared with the peak area of the (PAA/PAH)\textsubscript{6} multilayers. The functionalization of Fe\textsubscript{3}O\textsubscript{4} onto PAM\textsubscript{g} showed a larger effect at 3800-2300 cm\textsuperscript{-1} than that of PAM\textsubscript{g}GO. Additionally, the characteristic stretching vibrations of the C-O bond of GO nanosheets at 1050 cm\textsuperscript{-1}\textsuperscript{1103} were not detected (Figure 14E), while the characteristic C=O and O-H stretching vibrations of GO at 1740 cm\textsuperscript{-1} and 3400 cm\textsuperscript{-1}, respectively, overlapped with those of the PEMs. The intensity of the XRD pattern of the GO stacking layer on the (PAA/PAH)\textsubscript{6} multilayers was detected at 2\(\theta\) = 12.3\(^\circ\). This corresponded to an interlayer GO nanosheet spacing of 0.72 nm (Figure 14F) and further confirmed the presence of oxygen-containing functional groups. Furthermore, this may provide an explanation for the hydration phenomena observed with GO nanosheets in aqueous solutions.\textsuperscript{104} The surface charge density of PAH\textsubscript{g}GO may be higher than that of PAM\textsubscript{g}GO, thereby enabling more GO nanosheets to attach to the charged polymers. They were then detected through XRD measurement (arrow, Figure 14F). The XRD pattern was more evident for PAH\textsubscript{g}GO than for PAM\textsubscript{g}GO. The three sharp peaks at 2\(\theta\) = 16.9\(^\circ\), 18.9\(^\circ\) and 22\(^\circ\) are characteristic of PLLA (Figure 14F).\textsuperscript{105} The absorption peak of the Fe-O bond at 600-580 cm\textsuperscript{-1}\textsuperscript{1106} was not detected. These nanoparticles were likely physically adsorbed between multilayers at very low concentration. The surface grafting of AA monomers on the PLLA microspheres provided a denser and larger peak area of PEMs. The composition of these substrates is one of the crucial parameters to be considered during the assembly of the PAA/PAA\textsubscript{m} and PAA/PAH multilayers.
Table 5. Peak area of the two different substrates, i.e., non-grafted and PAA-grafted PLLA microspheres, after the adsorption of the sixth bilayer of PEMs.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Peak area region (A/cm)</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>v: 1800-1500 cm(^{-1})</td>
<td>v: 3800-2300 cm(^{-1})</td>
</tr>
<tr>
<td>PAM(_n)</td>
<td>18</td>
<td>8</td>
</tr>
<tr>
<td>PAH2.5(_n)</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>PAH5(_n)</td>
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<tr>
<td>PAH(_g)FeO</td>
<td>19</td>
<td>18</td>
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</tbody>
</table>

4.4.3 Morphological structures of PEMs

The effects of the adsorption of PEMs on microparticulate-based samples were evaluated using microscopy (Figure 15). The diameters of the microspheres were determined using Sauter’s method because the combined surface grafting and LbL approach resulted in an activated surface. For such systems, Sauter’s method is commonly used to calculate the diameter of microspheres. The assembly of the PEMs on two different base substrates affected the morphology of the microspheres. The neat PLLA microspheres showed a mean diameter of 2 \(\mu\)m (Table 6) and had a rough substrate surface (Figure 15A). The (PAA/PAAm)\(_6\) multilayers that were alternately adsorbed onto the PAA-grafted PLLA microspheres retained a rough structure similar to the neat PLLA microspheres (Figure 15B).
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Figure 15. Morphological structures of neat PLLA microspheres (A) and PAA-grafted PLLA microspheres after the assembly of (B) (PAA/PAAm)$_6$ multilayers at pH 2.5, (C) (PAA/PAH)$_6$ multilayers at pH 2.5, (D) (PAA/PAH)$_6$ multilayers at pH 5, (E) (PAA/PAAm/GO)$_6$ multilayers at pH 2.5, (F) (PAA/PAH/GO)$_6$ multilayers at pH 2.5, (G) (PAA/PAAm/Fe$_3$O$_4$)$_6$ multilayers at pH 2.5, (H) (PAA/PAH/Fe$_3$O$_4$)$_6$ multilayers at pH 2.5. The scale bar of the image indicated 1 µm.

In contrast to the grafted PLLA microspheres, for the non-grafted PLLA microspheres a smooth structure was revealed after the deposition of the PAA/PAAm multilayers (Figure 16A). Alterations in the surface morphology were observed for PAH2.5$_g$; the surrounding charges induced a conformational transition in the polyelectrolytes (Figure 15C). A nonstoichiometric ion pairing between these polycations and polyanions likely formed loopy and thick layers (Figure 15C), leading to a slightly larger diameter of approximately 11.5 ± 0.7 µm (Table 6). For the PAA-grafted PLLA microspheres, an electrostatic-driven mechanism (Figure 15C) resulted in a rougher surface than that of the hydrogen bonding-driven interaction at low pH (Figure 15B). Additionally, the alternating adsorption of PAA/PAH multilayers onto the grafted microsphere surfaces led to a better surface coating than the non-grafted analogues (Figure 16B). The changes in surface
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morphology indicated that the PEMs were successfully adsorbed onto the microsphere surfaces and thereby confirmed the surface chemistry analysis (Figure 14).

Figure 16. Morphological structures of non-grafted PLLA microspheres after the assembly of (A) (PAA/PAAm)$_6$ multilayers at pH 2.5, (B) (PAA/PAH)$_6$ multilayers at pH 2.5, (C) (PAA/PAH)$_6$ multilayers at pH 5. The scale bar of the image indicated 1 µm.

Conformational chain transitions of (PAA/PAH)$_6$ multilayers was seen when the pH of the solution was increased to 5. A smooth surface morphology was captured after alternatingly assembling (PAA/PAH)$_6$ multilayers onto the PAA-grafted PLLA surfaces at pH 5 (Figure 15D). It is worth noting that a rougher surface on the non-grafted PLLA microspheres was still observed when increasing the pH of the solution (Figure 16C), in contrast to what was found for the grafted PLLA microspheres (Figure 15D). The functionalization of nanomaterials into the multilayers, i.e., GO or Fe$_3$O$_4$, yielded a good surface coating on PAH2.5$_g$ and changed the surfaces morphology for PAH2.5$_g$ (Figure 15F and 15H) and PAM$_g$ (Figure 15E and 15G) at low pH. In addition, no significant alteration in surface morphology of PAA/PAAm multilayers and PAA/PAH multilayers was captured after GO functionalization (Figure 15E and 15F). Surface grafting hence affected the morphology of the microspheres during the assembly of the PEMs.

4.4.4 Mechanical performance of 3D microsphere scaffolds

The PEMs physically adsorbed on the grafted PLLA microspheres were packed into a 3D template in order to make scaffolds. The compressive modulus of the 3D particulate scaffolds was measured to evaluate the scaffolds mechanical performance in comparison to natural materials, e.g., cancellous bone. PAH2.5$_g$ demonstrated good mechanical performance with E-modulus of 141 ± 19 MPa, while PAM$_g$ had a slightly lower mechanical performance than PAH2.5$_g$ with E-modulus of 115 ± 5 MPa. These two 3D microspheres scaffolds, fabricated at low pH, showed higher Young’s modulus than the other scaffold, i.e., PAH5$_g$ (Figure 17A).
**Results and Discussion**

Figure 17. (A) Comparison of compressive Young’s modulus (E-modulus) of various 3D-microsphere scaffolds, (B) Ashby’s plot of E-modulus vs. density of natural materials in comparison with the synthetic 3D scaffolds, (C) An illustration of 3D microsphere scaffold.

Additionally, the functionalization with GO nanosheets with a zeta potential of -23 mV between the (PAA/PAAm)$_n$ multilayers improved the mechanical performance of PAM$_g$. This scaffold had an E-modulus level similar to that of the PAH2.5$_g$ scaffold at approximately 146 ± 6 MPa. After functionalization with the GO nanomaterials, the E-modulus of PAH2.5$_g$ decreased significantly to a very low mechanical performance, 16 ± 2 MPa. PAH5$_g$ showed a much lower mechanical performance than PAH2.5$_g$ with E-modulus of 22 ± 3 MPa. The conformational chains and thickness of the multilayers influenced the mechanical properties of these 3D materials. The 3D scaffolds of PAM$_g$, PAH2.5$_g$ and PAM$_g$GO had similar mechanical performances as 100-600-µm diameter microspheres sintered at 60-70°C$^{111}$ or 100°C for 4 h$^{60}$ or 160°C for 2 h$^{112}$. However, these 3D microsphere scaffolds showed a greater mechanical performance when compared with solvent/non-solvent microsphere scaffolds prepared via sintering.$^{113}$ An increase in the sintering temperature and time had detrimental effects on the morphology. Additionally, the porosity of the scaffolds was also reduced,$^{60,111}$ rendering the 3D scaffolds unsuitable
because porosity affects tissue regeneration. All 3D microsphere scaffolds fabricated via the surface grafting and LbL approach displayed mechanical performance levels similar to human cancellous bone, i.e., E-moduli of 10-2000 MPa. However, at medium density, the bone has E-moduli higher than 100 MPa (E-moduli > 100 MPa)\textsuperscript{114} according to Ashby’s plot (Figure 17B).\textsuperscript{115} Only three 3D microsphere scaffolds were within this range a natural human bone: PAH2.5\textsubscript{g}, PAM\textsubscript{g} and PAM\textsubscript{g}GO (Figure 17B). The 3D microsphere-based scaffolds prepared via surface grafting and LbL approach were successfully designed and later functionalized with GO nanomaterials (Figure 17C). They retained the good mechanical performance required for cancellous bone regeneration.

### 4.4.5 Physical properties of various microparticulate samples

The combined surface modifying technique (surface grafting and LbL approach) on PLLA microspheres increased the diameter and the thickness of the PEMs (Table 6). The dry thickness of the grafting layer ranged from 800 nm to 2.2 µm. The microspheres with alternately deposited (PAA/PAH)\textsubscript{6} multilayers or PAH2.5\textsubscript{g} showed a larger diameter of approximately 11.5 ± 0.7 µm, which was 4-5 µm larger than those with (PAA/PAAm)\textsubscript{6} multilayers or PAM\textsubscript{g}. The differences in their diameters could be due to the presence of a loopy conformation formed on the PLLA surfaces when assembling PAH2.5\textsubscript{g} (Figure 15C). The functionalization with the GO nanosheets changed the microsphere properties. When compared with PAH2.5\textsubscript{g}, PAM\textsubscript{g} increased in diameter and thickness and decreased in specific surface area per unit mass of PLLA microspheres after GO functionalization. Additionally, the mechanical performance of the PAH2.5\textsubscript{g} and PAM\textsubscript{g} scaffolds (Figure 17A and 17B) could be related to their microsphere properties, i.e., they had thicker PEMs layers, which improved their mechanical performance (Table 6). According to Ashby’s diagram, the 3D scaffolds of PAM\textsubscript{g}, PAH2.5\textsubscript{g}, and PAM\textsubscript{g}GO were within the high compressive moduli levels of cancellous bone (Figure 17B). The PAM\textsubscript{g} and PAM\textsubscript{g}GO scaffolds also had similar porosities as cancellous bone, i.e., 50-90\%,\textsuperscript{114} while the porosity of the PAH2.5\textsubscript{g} scaffold was high enough to meet the requirements for repairing femoral defects in rabbits.\textsuperscript{116}

During bone repair, external electric stimuli at minimum frequency from 10-30 Hz can provide a stimulus for new bone formation. Bone tissue responds to an electric signal between 1-10 µV/cm.\textsuperscript{64} Thus, the electrical conductivity of scaffolds plays an important role in bone regeneration. The conductivities of GO and Fe\textsubscript{3}O\textsubscript{4}-functionalized 3D scaffolds were measured.
Table 6. The diameter and surface area, layer thickness, and porosity of microparticulate-based samples.

<table>
<thead>
<tr>
<th>Type of sample</th>
<th>Microsphere properties</th>
<th>Layer thickness$^c$ (µm)</th>
<th>Porosity of 3D scaffold (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean diameter$^a$, d (µm)</td>
<td>$S_m^b$</td>
<td></td>
</tr>
<tr>
<td>PLLA</td>
<td>2</td>
<td>1.2</td>
<td>-</td>
</tr>
<tr>
<td>PLLA-g-PAA</td>
<td>3.5±0.7</td>
<td>0.6</td>
<td>1.5±0.7</td>
</tr>
<tr>
<td>PAH2.5$_g$</td>
<td>11.5±0.7</td>
<td>0.2</td>
<td>9.5±0.7</td>
</tr>
<tr>
<td>PAM$_g$</td>
<td>7</td>
<td>0.3</td>
<td>5</td>
</tr>
<tr>
<td>PAH$_g$GO</td>
<td>4</td>
<td>0.6</td>
<td>2</td>
</tr>
<tr>
<td>PAM$_g$GO</td>
<td>12</td>
<td>0.2</td>
<td>10</td>
</tr>
</tbody>
</table>

$^a$Calculated using Sauter’s method, $^b$Sm = Specific surface area per unit mass, $^c$Layer thickness of PEMs was calculated by subtracting the PEMs-adsorbed microspheres with the neat PLLA microspheres, $^e$ not possible to fabricate 3D scaffolds.

Without functionalization, the 3D scaffold did not show any electrical conductivity. The GO-functionalized 3D PAM$_g$ and PAH2.5$_g$ scaffolds were not able to conduct a 1 mA electric current. There may be a critical GO concentration required for conductivity; however, these GO-functionalized microspheres are electrical insulators.$^{117}$ The Fe$_3$O$_4$-functionalized 3D scaffolds had high electrical conductivity above 1 S/cm.$^{118}$ The electrical conductivity for the PAH2.5$_g$ and PAM$_g$ scaffolds were approximately 180 S/cm and 130 S/cm, respectively, although the levels of Fe$_3$O$_4$ in the scaffolds were low at 0.002 wt.%. Doping with Cl$^-$ ions resulted in significant changes in the electrical conductivity. When doped with Cl$^-$ ions, the electrical conductivity of PAH2.5$_g$ decreased by nearly sixty-fold to approximately 3 S/cm when compared with the non-doped scaffolds. However, the Cl$^-$ doping of PAM$_g$FeO scaffolds resulted in a much higher conductivity at approximately 240 S/cm than non-doped PAM$_g$FeO scaffolds. It is likely that a higher concentration of Cl$^-$ ions penetrated the (PAA/PAH)$_6$ multilayers. This decreased the electrical conductivity of the PAH$_g$FeO scaffold due to a redox reaction and also decreased the magnetism. The successful fabrication of 3D microsphere scaffolds via surface grafting and LbL approach showed that these scaffolding materials could be functionalized with nanomaterials while retaining their porous structures and mechanical performance at levels similar to human cancellous bone (Figure 17C).
4.5 THE EFFECT OF THE POLYMER GRAFTS AND SUBSTRATE SHAPE ON SURFACE DEGRADATION

The grafted microspheres have been shown to prevent agglomeration due to long-range repulsive forces. The grafting layers have also been used as base substrates to provide physical interactions with charged polymer layers. The PEM-coated microspheres were subsequently used for the fabrication of 3D scaffolds. The effects of the grafted chains on the stability of the particles in PBS for up to 30 days were shown in the differences observed in the surface morphology, chemistry, and topography of the microparticulate substrates relative to film substrates.

4.5.1 Morphologies of different PLLA substrates before, during and after surface degradation

The morphology of the film surfaces was more altered (Figure 18) when compared with the microparticulate surface (Figure 19) after immersion in phosphate buffered saline (PBS). Prior to PBS immersion, the surfaces of the PLLA-g-PAAm and PLLA-g-PAA substrates were smooth (Figures 18B and 18C), whereas the neat PLLA film revealed pores due to solvent evaporation during solvent-casting (Figure 18A). For the PLLA-g-PAA films, macrocracks formed after day 10 of PBS immersion (Figure 18F); these cracks remained visible throughout the remainder of immersion (Figure 18I). For the neat PLLA films, smoother surfaces than PLLA-g-PAA films were observed on the surface after 14 days of PBS immersion (Figure 18D) and were retained after 30 days of immersion (Figure 18G). The neat PLLA microparticles displayed consistent surface morphologies before (Figure 19A), during (Figure 19D), and even after a month of PBS immersion (Figure 19G). Similarly, the surface morphologies of the film and microparticulate substrates with PAAm grafts did not change after 30 days of PBS immersion (Figures 18H and 19H). Additionally, when AA was combined with AAm in the polymer grafts, no significant differences were also observed on the film substrate throughout the entire degradation period.
For the microparticles, the formation of macrocracks due to the presence of PAA grafts were not visible. Instead, rough surfaces were observed on the microparticles after 30 days of surface degradation (Figure 19I). Additionally, for surfaces grafted with a combination of two hydrophilic monomers, AA and AAm, the morphology of the substrates changed slightly after 30 days of surface degradation.
Results and Discussion

Figure 19. The morphology of microparticles of (top): (A) PLLA, (B) PLLA-g-PAAm, and (C) PLLA-g-PAA at day 0; (middle): (D) PLLA, (E) PLLA-g-PAAm and (F) PLLA-g-PAA at day 14; (bottom) (G) PLLA, (H) PLLA-g-PAAm and (I) PLLA-g-PAA at day 30. The scale bar indicated 10 µm.

As previously noted, PBS immersion at pH 7.4 affected the surface morphology of the PLLA-g-PAA films. To determine whether these macrocracks were due to the actual PAA grafts attached to the PLLA backbone or were a natural response to the acidic environment created by the PAA chains, neat PLLA films and microparticles were immersed in 0.3% (w/w) and 3% (w/w) PAA solutions, yielding pH values of 6.9 and 5.0 in PBS, respectively. These concentrations were used to determine whether the covalently attached PAA chains on the PLLA backbone were important. Unfortunately, the calculation of the exact amount of covalently attached PAA grafts to the surface of the PLLA films was not possible. However, as was previously shown, the maximum density of polymer grafts on the film substrates remained lower than the smallest amount of PAA dissolved in PBS solutions. At high pH, the PAA chains ionized due to the presence of acid groups, thereby releasing protons. It is already known that when this mechanism occurred during the PLLA degradation, it
later induced an autocatalytic effect and eventually accelerated the degradation of the main PLLA chain. No alterations on the neat PLLA film substrates were observed even after 30 days in the highly acidic solution (Figure 20). This is in contrast to the phenomenon observed on the surface of the PLLA-g-PAA films, where damaging macrocracks were visible as early as day 10 of PBS immersion (Figure 18F).

The observed differences in surface morphologies between the PAA-grafted PLLA films and the neat PLLA films in a solution consisting of PAA chains (which were similarly treated using the same degradation procedure as the grafted films) was caused by a localized acidic environment created by the covalent attached polymer grafts. As was observed in the PLLA film substrates, no alterations in surface morphologies were detected when immersing these PLLA microparticles in a highly acidic solution (Figure 21).

**Figure 20.** The morphology of neat PLLA films of (top): (A) in 0.3 % (w/w) PAA solution, (B) in 3 % (w/w) PAA solution at day 14; (bottom) (C) in 0.3 % (w/w) PAA solution, (D) in 3 % (w/w) PAA solution at day 30.
Results and Discussion

Figure 21. The morphology of neat PLLA microparticles of (top): (A) in 0.3 % (w/w) PAA solution, (B) in 3 % (w/w) PAA solution at day 14; (bottom) C) in 0.3 % (w/w) PAA solution, (D) in 3 % (w/w) PAA solution at day 30.

When the PAA chains were covalently attached to the PLLA substrates, the resultant localized acidic environment altered the surface morphology to a greater extent than in the neat PLLA and the PLLA substrates with covalently attached PAAm.

4.5.2 Evaluation of surface chemistry on different geometrical substrates

The surface chemistries of the PLLA films and microparticulate substrates at certain time points during PBS immersion were also evaluated using FTIR (Figure 22). As expected, no significant changes to the PLLA references were observed (Figure 22A). Due to the overlapping C=O characteristic bands between PLLA and PLLA-g-PAA at approximately 1747 cm\(^{-1}\), there were no detectable alterations in the surface chemistries of the PAA-grafted PLLA films and microparticles when immersed in PBS solution from day 0 to day 30 (Figure 22D).
Results and Discussion

Figure 22. ATR-FTIR spectra of both films and microparticles of (A) PLLA, (B) PLLA-g-PAAm, (C) PLLA-g-PAA/PAAm, (D) PLLA-g-PAA, (E) and (F) peak area of PAAm and PAA/PAAm shown in A/cm and in percentage, respectively, at 1700-1550 cm\(^{-1}\) upon PBS immersion times of 0 (a), 5 (b), 10 (c), 14 (d) and 30 (e) days.

‘Bumps’, or double absorption bands, were observed on the PLLA backbone after 1.5 h of grafting, when grafting AAm on the surface of the PLLA substrate. Furthermore, the characteristic amide I band at 1660 cm\(^{-1}\) and the absorption band of the primary
amide (amide II band) at 1615 cm$^{-1}$ were visible (Figure 22B). The shape of the substrates seemed to affect the surface grafting process because more PAAm chains were attached to the PLLA backbone of the films than to the microparticles, resulting in a larger amide peak area for the PLLA films (6 A/cm$^2$) than that for the PLLA microparticulates (3 A/cm$^2$). However, when both films and microparticles were surface-grafted with a mixture of AA and AAm with a volume ratio of 1:1 (Figure 22C), a larger peak area at approximately 7 A/cm$^2$ was observed for the microparticles than for the films (3 A/cm$^2$; Figure 22E). After immersion in a salt-based water solution for 5 days, the amount of PAAm grafts was significantly reduced for both substrates (Figure 22E). A peak area reduction of approximately 40% was observed for the PLLA-g-PAAm films, whereas an approximately 60% reduction in the peak area of the PLLA-g-PAAm microparticles was observed (Figure 22F). No alterations in morphology of the two substrates were detected (Figures 18H and 19H), even though the peak area of the PAAm-grafted films significantly decreased after 5 days of PBS immersion. Between days 14 and 30 of PBS immersion, the amount of PAAm grafts observed on the microparticles by FTIR did not change, while the amount of the same polymer grafts on the films decreased. The loss of PAAm chains occurred on the surfaces up to day 30 when the peak area remained at approximately 16% (1 A/cm$^2$) for the films and 24% (0.8 A/cm$^2$) for the microparticles (Figure 22E). Additionally, at day 30, the PAAm grafted microparticles had larger peak areas than those of the films. This may be due to the compression of the grafted chains against each other resulting in a higher grafting density; their existence was detected by FTIR spectroscopy. After 10 days of PBS immersion, more polymer grafts seemed to detach from their microparticulate surfaces (Figure 22E). Osmotic and entropic elastic driving forces could play significant roles in the compression of the grafted chains; these forces might have reached an equilibrium. The phenomenon is most likely more complex when using two different hydrophilic monomers, i.e., AA and AAm. The FTIR spectra of the PLLA-g-PAA/PAAm microparticles (Figure 22C) exhibited similar trends as those of the PLLA-g-PAAm microparticles (Figure 22B). The polymer grafts degraded much faster from the surfaces of the PLLA microparticles than from the films at day 5. However, the presence of the PAA/PAAm grafts affected their stability on the PLLA microparticle surfaces, decreasing the peak area to approximately 20%, whereas a peak area of 40% for the PAAm grafts remained (Figure 22F). In summary, the surface degradation of the polymer grafts on the surface was observed. While a relatively low amount of grafted chains was detected by FTIR spectroscopy at day 30 for both different substrates, a layer of polymer graft remained. Previously, when PLLA films were surface-grafted with PAA, the PAA-grafted PLLA films were solubilized in chloroform and subsequently, their $M_n$ was determined by SEC. While the FTIR spectra demonstrated a very weak intensity from the PAAm grafts, the grafts remained covalently anchored on both substrate surfaces after 30 days of PBS immersion;
Furthermore, the samples could not be dissolved. However, the neat PLLA references were soluble in chloroform. This phenomenon indicates that after 30 days of PBS immersion, the grafting layer sufficiently prevented dissolution and that this was not attributed to cross-linking reactions.

4.5.3 Topographies of different PLLA substrates before and after surface degradation studies

To further investigate the effects of polymer grafts on the surface degradation of PLLA-based substrates, the roughness of these substrates before and after surface degradation were calculated (Table 7). The topographies of the substrates were dependent on the polymer graft and the shape of the substrate. For the grafted substrates, the surface roughness was much larger for the films than for the microparticles. Furthermore, the larger detrimental effect on the surface topography was confirmed for PLLA substrates grafted with AA monomer than with AAm monomer. For the PLLA substrates, the surface roughness generally decreased up to 30 days PBS immersion, whereas the opposite profile was observed for the grafted surfaces. The largest changes in surface roughness were observed for the PLLA-g-PAA films (Figure 18F and 18I), as indicated by the sizeable macrocracks after only 10 days. Initially, a rough surface (Rq = 21 ± 1 nm) was observed for the neat PLLA films (Table 7) after UV-irradiation in ethanol. The surface roughness of the PLLA films was slightly reduced after 30 days PBS immersion (Rq = 16 ± 5 nm). The same roughness trend was also observed for the PLLA microparticles, where a rougher surface was initially detected after UV-irradiation (Rq = 30 nm) when compared with the roughness after 30 days surface degradation (Rq = 7 nm).

Table 7. Surface roughness (Rq) of PLLA films and microparticles during surface degradation at days 0 and 30.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Surface roughness (nm)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Film</td>
<td>Microparticle</td>
</tr>
<tr>
<td></td>
<td>Time (day)</td>
<td>Time (day)</td>
</tr>
<tr>
<td>PLLA</td>
<td>21±1</td>
<td>16±5</td>
</tr>
<tr>
<td>PLLA-g-PAA</td>
<td>22</td>
<td>125±20</td>
</tr>
<tr>
<td>PLLA-g-PAAm</td>
<td>6</td>
<td>11±1</td>
</tr>
</tbody>
</table>
When the hydrophilic AA monomer was covalently grafted on the surfaces of the films and particles, the resultant surface roughnesses were different (Table 7). The PLLA-g-PAA microparticles had smoother surfaces than the films after UV-irradiation. After 30 days of PBS immersion, the PLLA microparticles had a surface roughness thirteen-fold rougher than before, whereas the topography of the film substrates was approximated to be six-times rougher than the initially calculated values prior to surface degradation. The PLLA-g-PAA film demonstrated the roughest topography when compared with all PLLA-based films after PBS immersion.

The topographies of the films and microparticles grafted with PAAm before PBS immersion exhibited the opposite trends for the rms roughness, i.e., the surface roughness decreased and became smoother relative to the PAA-grafted PLLA surfaces. Additionally, the rms roughness did not demonstrate any significant changes for the microparticulate substrates after 30 days immersion, whereas the PLLA films had a two-fold rougher surface (Table 7). These topographic results are thus well-fitted to the observed changes in the morphological structures. The PAA grafts ionized due to a high pH, leading to the creation of a localized acidic environment, the degradation of the PLLA film surfaces at days 14 and 30 (Figures 18F and 18I) and the increased roughening of PLLA microparticles after 30 days (Figure 19I).

4.5.4 Evaluation of molecular weight during surface degradation

No significant changes in the \( M_n \) were observed for the non-grafted surfaces before and after 30 days PBS immersion (Table 8).

**Table 8.** The number average molecular weight \( (M_n) \) of PLLA films and microparticles before and after surface degradation as a function of time.

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>( M_n (g.mol^{-1}) )^a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Film</td>
</tr>
<tr>
<td></td>
<td>PLLA</td>
</tr>
<tr>
<td>0</td>
<td>124,000</td>
</tr>
<tr>
<td>5</td>
<td>132,000</td>
</tr>
<tr>
<td>10</td>
<td>129,000</td>
</tr>
<tr>
<td>14</td>
<td>126,000</td>
</tr>
<tr>
<td>30</td>
<td>122,000</td>
</tr>
</tbody>
</table>

^a Determined by SEC analysis (polystyrene standards for calibration and chloroform as an eluent)
All grafted substrates were insoluble in the organic solvents used for SEC characterization, indicating that the PAA or PAAm grafting layers remained strongly anchored on the substrate surfaces. The $M_n$ of neat PLLA films and microparticulates, during and after the surface degradation study, were similar to their initial values before UV-irradiation and material fabrication (Table 8). This confirmed the non-destructive grafting method and that the bulk did not degrade.
5 CONCLUSIONS

A covalent ‘grafting-from’ technique was successfully designed and applied to PLA particle surfaces. The technique was shown to be non-destructive as indicated by retained molecular weights and 3D shapes of the substrates. Changes to the surface chemistries of the particles confirmed the success of the surface grafting technique. Additionally, the changes to the surface morphologies of the particles also indicated the success of the ‘grafting-from’ technique.

Particle aggregation is prevented and colloidal stability is achieved when the surfaces of colloidal particles are covered with grafting layers. Hydrophilic polymers that were covalently attached to the surfaces of PLA substrates exhibited long-range repulsive interactions useful for overcoming particle aggregation. In other words, aggregation was hindered due to steric interactions between two interacting grafted surfaces. Purely steric interactions were observed in a few symmetrical systems, such as neutral/neutral polymer grafts and pH sensitive/pH sensitive polymer grafts, and the asymmetrical system of non-grafted polymer/neutral polymer grafts.

Furthermore, the grafted surface of microspheres was used as a base layer for alternating assembly of weak polyelectrolytes via LbL approach. The combined surface treatment was later used to design 3D microsphere scaffolds suitable for bone tissue engineering. The growth of the PEMs on PAA-grafted particles was determined by distinct changes in the zeta potentials and by alterations in their surface chemistry. During the formation of the PEMs, the different substrates controlled the morphology and density of the grafted functional groups on the microsphere surfaces. The functionalization with nanomaterials significantly changed microsphere properties, such as substrate thicknesses and physicochemical properties of 3D scaffolds such as mechanical performances and electrical conductivities. Functional GO nanosheets were incorporated within the multilayers and thereby improved the mechanical performance of polyanion and neutral polymer multilayers. The magnetic nanoparticles-functionalized 3D scaffolds showed high electrical
conductivity; doping reduced the electrical conductivity of these particles within polyanion and polycation multilayers but not within polyanion and neutral polymer multilayers. The mechanical performances of all 3D scaffolds were within the ranges reported for human cancellous bone; furthermore, these scaffolds had suitable porosities required for bone tissue regeneration.

These hydrophilic polymer grafts and substrate geometries were shown to affect the surface degradation of PLLA films and microparticles. The different properties of polymer grafts, i.e., acid and pH-sensitive polymers as compared to neutral polymers, altered surface morphologies and chemistries. Based on the morphology, the acid and pH-sensitive polymer grafts induced greater changes on film substrates than on microparticles. However, neutral polymer grafts did not show any detrimental effects on the surface morphology of either geometry during the month-long degradation assays. Based on these evaluations, neutral polymer grafts demonstrated more rapid surface degradation rates from microparticle surfaces than from film surfaces in the early stages of degradation.
6 Future work

This thesis presented four different goals, including the development of surface grafting techniques to the fabrication of 3D microsphere scaffolds usable in bone tissue engineering. Future works in these areas could be explored as follows:

- It would be of interest to determine the properties of end-grafted polymers attached to microsphere surfaces, such as the chain length and molecular weight.
- The effects of in vitro and in vivo environments on 3D microsphere scaffolds could be further explored to evaluate cellular activities, determine the degradation rates and to evaluate the physicochemical properties of these particulate-based scaffolds.
- Further in-depth characterizations of the properties of the polyelectrolyte multilayers on the surfaces of PAA-grafted PLLA microspheres for each adsorption step could be explored, i.e., the charge density, the conformation of polyelectrolytes, and the swelling dynamics of each layer.
- Other future works should be focused on the amount of end-grafted layers remaining before and after 30 days surface degradation for films and particles. A separate surface degradation study could focus on longer degradation periods to determine whether larger surface alterations on the PLLA microparticles would occur.
ACKNOWLEDGEMENTS

First of all, I would like to express my gratitude to my principal supervisor, Professor Ann-Christine Albertsson, and my co-supervisors, Dr. Karin Odelius and Dr. Anders Höglund, for providing the opportunity to be a part of the Polymer Technology group and for this challenging PhD project. I would also like to convey my gratitude for their guidance, critiques, and encouragement throughout these tough years.

The ERC Advanced Grant PARADIGM (Grant Agreement No: 246776) is acknowledged for their financial support.

All senior scientists and PhD students at the Polymer Technology group are also recognized for their help and comfortable working atmosphere. I especially would like to thank the administrative officers, Mrs. Inga Persson and Mrs. Vera Jovanovic, for their warmth, attention and kindness. You were always ready when I needed urgent help. Karin Backström and Kjell Agnekil are also acknowledged for their kind help in IT problems. Mr. Bosse Swensén is acknowledged for his aid in troubleshooting technical issues with lab instruments.

I would also like to thank Dr. Torbörn Pettersson and Professor Lars Wågberg for the in-depth discussions during my doctoral study. Special thanks are also due to my collaborator, Weifeng Zhao, for the collaborative work on stimuli-responsive hemicellulose. I would also like to convey my gratitude to Dr. Necati Harmankaya for helping with fluorescence microscopy, Dr. Changgang Xu for nanomaterial preparation and analysis, Dr. Yang Sun for discussions and literature search with regards to µCT. The duo Dr. ‘J+M’ are thanked for their jokes and discussion about T.O.E. topics. I agree with one of you said that we should do what we think is best and do not care about other people’s perceptions because they sometimes do not understand what we think. Dongming Liu is acknowledged for his help with TEM. I would like also to convey my gratitude to Huiran Lu for her help in measuring electrical conductivity. Dr. Jenny Undin and Dr. Anders Höglund are thanked for ‘Sammanfattning’ of the thesis. Assya and Weifeng are thanked for comments and
corrections for this thesis. Additionally, big thanks are due to the people I have met in and outside the FPT department during my PhD studies. Everything has been fun, friendly and nice with you guys: Dr. MC², Dr. N², Mrs. HB and family, Mr. GR and family, YZR, her family and lovely mom, ‘The R.A.W’ gang, ‘big guy’ at the division of immunology at KI, a nice D guy and his couple, Haj & Hajja AA, JCF, XCG, AGC, AGG, king Arthur, B², Mr. B and his crazy gang+his lovely bro, and Mr. Chef and his couple. To the other individuals and groups I may have not mentioned, ‘thank you so much for your warmth, hospitality and kindness’.


Thank you! Terima kasih!
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Paper II
Paper III
Paper IV