

Hierarchically Structured Polymer Surfaces with Nanoparticles

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- Provést základní experimenty přípravy strukturovaných povrchů na bázi polystyrenu nebo polykaprolaktonu a deponovat na ně vybrané nanočástice.
- Provést důkladnou analýzu povrchů pomocí mikroskopických a spektroskopických metod, a dále zhodnotit vliv různých typů procesních parametrů.
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ABSTRAKT

Tato diplomová práce se zabývá přípravou a analýzou hierarchicky strukturovaných polymerních povrchů na bázi polystyrenu, které jsou určeny pro potenciální využití v tkáňovém inženýrství. Hlavním cílem bylo kombinovat mikroměřítkové topografie, vytvořené pomocí tepelného smrštění (tzv. wrinkling), s nanoměřítkovými strukturami tvořenými nanočásticemi polydopaminu (PDA), které byly nanášeny in-situ i ex-situ metodami. Byly zkoumány dvě strategie úpravy povrchu: (i) přímé tepelné strukturování psPS substrátů následované imerzí do alkalické reakční směsi pro syntézu PDA a (ii) nanášení vrstev biopolymerních filmů z lysozymových nanovláken (LNF) a kyseliny hyaluronové (HA) metodou spin-coating, jejich následné zvrásnění a modifikace PDA.

Morfologie povrchu byla hodnocena především pomocí rastrovací elektronové mikroskopie (SEM), zatímco další charakterizace byla provedena pomocí FTIR spektroskopie, dynamického rozptylu světla (DLS) a cirkulárního dichroismu (CD). In-situ depozice PDA na zvrásněné psPS substráty vedla za optimalizovaných podmínek k rovnoměrnému pokrytí. Multivrstvé LNF-HA filmy usnadnily tvorbu vrásek a sloužily jako funkční substráty pro následnou modifikaci PDA, přičemž chemické složení a morfologie povrchu závisely na typu vrchní vrstvy. Ex-situ syntéza PDA ukázala vývoj velikosti částic v čase a potvrdila schopnost vytvořit souvislý film s typickou zvrásněnou morfologií po delším stárnutí. Tato práce představuje dostupný a účinný způsob přípravy strukturálně i chemicky laditelných povrchů a přináší cenné poznatky pro budoucí výzkum v oblasti biorozhraní a tkáňového inženýrství.

Klíčová slova: biopolymery, polydopamin, hierarchické modifikace, spin-coating, SEM

ABSTRACT

This thesis presents the fabrication and analysis of hierarchically structured polystyrene-based polymer surfaces designed for potential applications in tissue engineering. The primary goal was to integrate micro-scale topographies, introduced via thermal wrinkling, with nano-scale features formed by polydopamine nanoparticles (PDA NPs) deposited through both in-situ and ex-situ approaches. Two modification strategies were explored: (i) direct wrinkling of pre-stressed polystyrene (psPS) substrates followed by immersion in a dopamine-based reaction solution, and (ii) spin-coating of biopolymer films composed of lysozyme nanofibers (LNF) and hyaluronic acid (HA), followed by wrinkling and PDA deposition. Surface morphology was primarily assessed using scanning electron microscopy (SEM), while additional characterization was performed using Fourier-transform infrared spectroscopy (FTIR), dynamic light scattering (DLS), and circular dichroism (CD). In-situ deposition on wrinkled psPS substrates yielded uniform PDA coverage under optimized conditions. Multilayer LNF-HA coatings facilitated wrinkling and served as functionalizable substrates for PDA deposition, with surface adhesion and morphology dependent on the outermost layer. Ex-situ PDA synthesis revealed size evolution of nanoparticles and confirmed their potential to form continuous films with wrinkled morphology after prolonged aging. This work demonstrates a feasible route for the generation of structurally and chemically tunable surfaces using accessible methods, with implications for future studies targeting cell adhesion or biointerfaces.

Keywords: biopolymers, polydopamine, hierarchical modification, spin-coating, SEM

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INTRODUCTION

Polymeric materials offer vast opportunities for surface engineering, enabling the development of tailored micro- and nano-scale features critical to numerous applications, including biomedical devices, sensors, and tissue scaffolds. One strategy for enhancing surface functionality is the fabrication of hierarchical structures that combine distinct length scales. These multiscale surfaces can influence biological responses, such as protein adsorption and cellular adhesion, making them particularly relevant for tissue engineering.

(1)

This thesis focuses on the modification of pre-stressed polystyrene (psPS) substrates to obtain hierarchical surface structures via two main pathways: thermal wrinkling and nanoparticle deposition. Thermal wrinkling was achieved by shrinking the psPS under controlled heating conditions, resulting in micron-scale surface undulations. Nanoscale features were introduced using polydopamine nanoparticles (PDA NPs), leveraging the material's robust adhesion and bioactivity.

The objective of this thesis is to develop and evaluate hierarchical polymer surfaces that integrate micro- and nano-scale structural features for potential use in biomedical applications. To achieve this, lysozyme nanofibers (LNF) and hyaluronic acid (HA) were employed as biopolymeric coatings applied via spin-coating onto psPS substrates. These biopolymers were selected for their complementary surface charges and biological relevance. Multilayer LNF-HA films were thermally wrinkled to create microstructured surfaces, which were then functionalized with PDA NPs using both in-situ immersion and ex-situ spin-coating of pre-aged PDA suspensions.

Throughout this thesis, the samples were characterized using scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), dynamic light scattering (DLS), and circular dichroism (CD). The combination of accessible fabrication methods and systematic evaluation provided insights into the relationship between layer composition, surface structure, and nanoparticle adhesion. The findings offer a promising foundation for designing advanced biomaterials with tailored surface properties for future research in tissue engineering and related fields.

I THEORETICAL PART

1 POLYMERS

Polymers are long, chain-like macromolecules comprised of numerous covalently bonded units known as monomers. When the monomers that form a polymer chain are identical, we refer to them as homopolymers. Conversely, if a polymer molecule contains more than one type of monomer, it is termed a copolymer. (2)

The number of bonds that a monomer can form, referred to as functionality, is determined by the number of reactive double bonds or functional groups. The monomer's functionality dictates whether the molecule is strictly linear or if additional groups or side chains can attach to the main chain, potentially leading to the creation of a three-dimensional network. (2)

Via polymerization, the synthesis of polymers occurs when monomers bind together, forming a mixture of molecules with different molecular weights. Unlike low-molecular-weight compounds, polymer molecules typically do not exhibit exact characteristics due to the difficulty of controlling the polymerization process. However, a narrower molecular weight distribution can be achieved by adjusting the manufacturing process. Factors such as molecular weight, monomer sequencing, steric arrangement, side chains, and others can vary among molecules, and their distribution determines the resultant properties. Consequently, describing their physico-chemical characteristics with high precision may be challenging. Although their properties change with increasing molecular weight, the variation diminishes as molecular weight increases, as illustrated in the graph in Figure 1. Overall variability in their structure, modifiability, low density, and cost-effectiveness provide polymers with a range of properties, enabling their use across a broad spectrum of applications. (2)

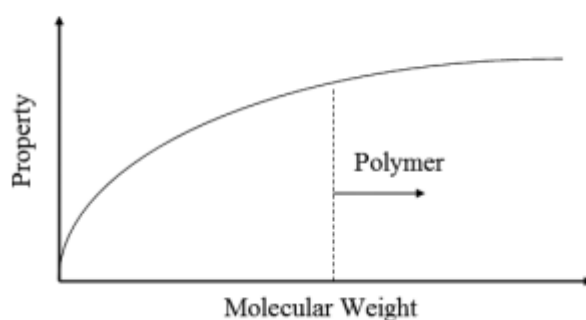


Figure 1 Several properties of polymers increase rapidly at low molecular weights and asymptotically approach a plateau due to chain entanglement effects (2)

1.1 Introduction and history of polymer

There are many naturally occurring polymers essential for life, such as nucleic acids, proteins, and polysaccharides. Natural polymers can be found almost everywhere in nature. In the mid- 19 th century, the first semi- synthetic polymer, nitrocellulose, was created by treating cotton with nitric acid. At the beginning of the 20th century, scientists began developing new materials by binding low-molecular substances and noted their interesting mechanical and rheological properties, such as high viscosity, non-Newtonian flow, low density, and good durability. The first synthetic polymer, Bakelite, was a resin prepared from the condensation of phenol and formaldehyde. This initial invention of a synthetic polymer was followed by a period of empirical exploration in the development of new polymers. In the 1950s, an era of synthetic polymers began, leading to rapid growth in the polymer industry. Polymers found applications in areas where wood, ceramics, or metals had previously dominated, including the machine industry, construction, engineering, and medicine, allowing other industrial sectors to emerge. Polyethylene, polypropylene, polyesters, polyurethanes, and polystyrene are the most widely used due to their excellent mechanical properties and low cost. The primary source of monomers for synthetic polymer production is petroleum, which is distilled into fractions, with naphtha, composed of molecules with 5 to 11 carbons, subjected to pyrolysis. This process retrieves unsaturated molecules, with ethylene, propylene, and butadiene being the most crucial monomers for the petrochemical industry. (2, 3)

Over the past few decades, both synthetically prepared and naturally derived polymers with biological characteristics have gained immense popularity in fields such as medicine and the pharmaceutical industry due to their degradability and biocompatibility. (2) Notable examples of biopolymers that have been extensively studied and utilized include proteins from soy or silk, collagen, gelatin, or polysaccharides such as starch, chitosan, and hyaluronic acid derivatives. (4) Polymers of natural origin have the advantage of being degradable, and with the recent trend and pressure for a “greener”, zero-waste and circular economy, many companies and researchers have started to study and incorporate biopolymers in their products. Biopolymers have been employed on their own or in composites as coatings or films, membranes, patches, nanosystems or microneedles. (5) Despite their great potential and obvious advantages, there are challenges presented when working with natural polymers, such as poorer mechanical and barrier properties or faster degradation, which can be both desired and unwanted depending on the specific use and

timeline of intended use. Some of the challenges can be solved by creating composites of biopolymers, either with another biopolymer, but in the form of micro- or nanoparticles, or in combination with a synthetic material.

1.2 Structure-Property Relationships in Polymer

This chapter presents some molecular structure principles fundamental to understanding key polymer properties.

1.2.1 Molecular structure

Bonds in polymers can be intramolecular – typically primary covalent bonds forming the main chain or backbone. The most common are C–C bonds, but others include C–N, C–O, C=C, O–O, Si–O, and C≡N. Side chains can also involve atoms like sulfur (S), silicon (Si), fluorine (F), and chlorine (Cl). The mean bonding length is approximately 1.5 Å, with a bond energy around 100 kcal/mol. (2)

Segments and molecules interact through intermolecular forces, which are weaker secondary interactions. These have typical lengths of about 3 Å and energies around 5 kcal/mol. They include hydrogen bonds and van der Waals (vdW) interactions. (2)

Van der Waals interactions include dispersion (London) forces, which arise from momentary fluctuations in the electron cloud, leading to instantaneous polarization. These are the weakest interactions and are common in apolar, aliphatic polymers like polyolefins. For example, polyethylene (PE) remains solid at room temperature due to these interactions. (2)

Stronger interactions include permanent dipole interactions (Keesom forces), observed in polymers with polar groups along the chain. In polyacrylonitrile (PAN), for example, nitrile groups (C≡N) on neighboring chains align side by side so that the CN dipoles can align in positions that compensate for the imbalance of charges. This alignment enables partial crystallisation and increases glass transition and melting temperatures. (2)

Permanent dipole–induced dipole interactions (induction forces, Debye forces) are not significant in polymers because if there is a permanent dipole in its monomers, they will interact with each other and so permanent dipole–permanent dipole interactions dominate. (2)

Hydrogen bonds form between a hydrogen atom and a small, highly electronegative element such as oxygen or nitrogen. In polymers, this interaction is typically observed in polyamides, where the amide linkage ($-\text{CO}-\text{NH}-$) allows hydrogen from the amide group to interact with the carbonyl oxygen of a neighboring segment. By adjusting the number of methylene ($-\text{CH}_2-$) groups between successive amide bonds, the spatial distribution and density of hydrogen bonds can be modulated. This directly affects the strength of intermolecular attraction between polymer chains or segments. As a result, the melting temperature of polyamides can be tuned to fall between that of simple polyethylene (nonpolar) and polyacrylonitrile (PAN), which has a high density of polar nitrile groups and only one methylene group per repeating unit, leading to a greater concentration of polar interactions and thus stronger intermolecular forces. (2)

The arrangement of monomers bound by strong primary bonds determines the material's chemical and thermal stability, while weaker secondary forces, which affect the relative positioning and packing of macromolecules and their segments, are primarily responsible for physical properties such as melting temperature, crystallinity, solubility, diffusion, permeability, deformation, and flow. (2)

Monomers with a reactive double bond, such as ethylene, or with two reactive groups, such as glycols, enable the growth of a polymer chain. Adding polyfunctional monomers enables the attachment of another group or polymer branch, possibly leading to cross-linking between the chains and forming a 3D network. The side chains—or branches—can be of the same monomers as the main chain or different. The branches may be arranged irregularly (randomly) in terms of length and distribution, or regularly, forming a comb-like structure. They can even be designed to attach to one polyfunctional centre to create a radial star-like shape. (6) The presence of a network particularly affects the material's rheological and thermal behaviour. Cross-linked polymers, termed thermosets, stiffen after passing the gel point during curing, which prevents them from melting when heated. A similar principle applies to rubbers produced by vulcanisation, where di-sulphuric bridges link neighbouring chains, although their cross-link density is lower than in thermosets. (2)

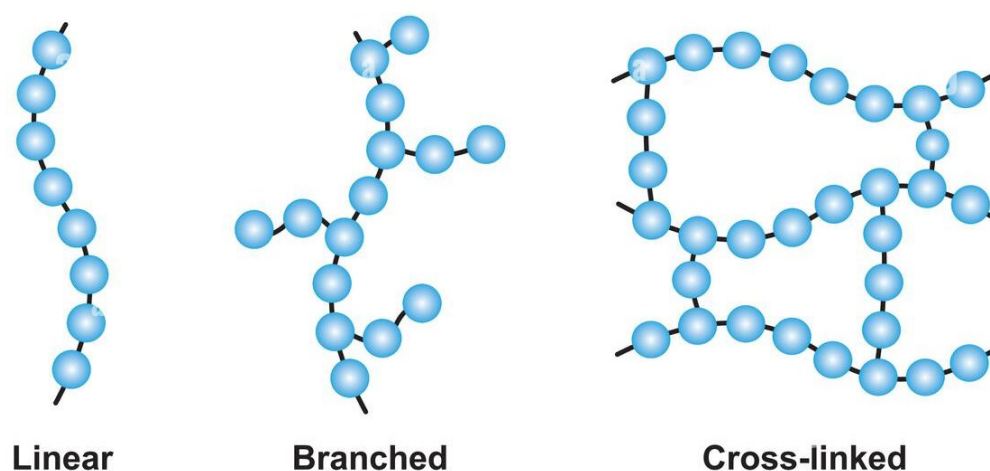


Figure 2 Schematic representation of different polymer structures (7)

Copolymers, like poly(ethylene terephthalate), which consist of randomly arranged monomers, gain properties intermediate between the two component polymers and have low to no crystallinity. Much more difficult to obtain, and thus quite rare, is a copolymer composed of alternating monomers, such as styrene maleic anhydride. Another arrangement involves alternating entire sequences of monomers (blocks) of varying lengths (e.g. AAA-BBBBBB-AAA), where A and B represent different types of monomers. An example is a styrene–butadiene triblock copolymer. Finally, graft copolymers contain a polymer chain covalently bound to a main homopolymer chain, e.g. homopolybutadiene grafted with a styrene–acrylonitrile copolymer. (2)

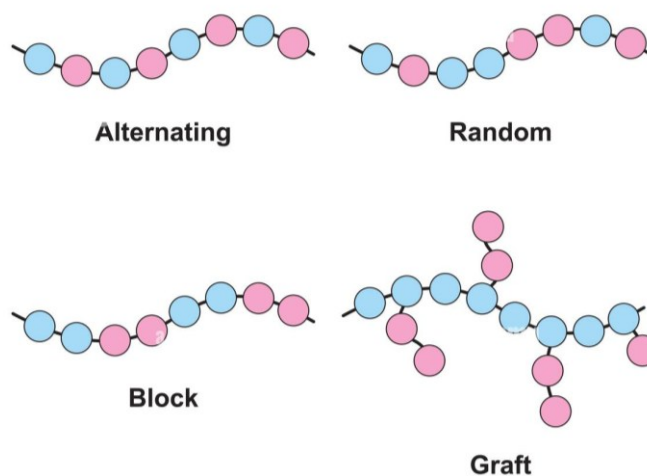


Figure 3 A schematic structure of different types of co-polymers (8)

1.2.2 Thermal behaviour

Thermoplastics are moldable because they flow when subjected to high temperature and pressure and solidify after normal conditions return. Due to their linear or branched molecular structure, it is possible to repeat the melting-solidifying process and to dissolve them. (2)

Polymers with linear or branched molecules flow when subjected to high temperatures, pressure or both and solidify after normal conditions return. This process can usually be repeated without major changes in the materials, enabling their moulding and recycling. Due to their behaviour when processed, they are called thermoplastics or baroplastics, depending on the prevailing condition causing the change. Their molecular structure further allows them to be dissolved. (2)

On the other hand, thermosets (or resins) are polymers that flow when heated and generate cross-links between their chains, taking on the form of the mould for the rest of their lifespan, becoming insoluble and non-recyclable. (3)

Polymers experience key thermal transitions that define their usability and processing. The most critical is the glass transition temperature (T_g), marking the shift from a rigid, glassy state to a soft, rubbery state. Semicrystalline polymers also exhibit melting temperature (T_m), corresponding to the crystalline domain's disruption. (3)

The nature and frequency of side groups, molecular symmetry, and chain rigidity all influence T_g and T_m . Bulky or stiff side groups (e.g. phenyl) hinder chain mobility and raise T_g , while flexible backbones lower it. For applications involving thermal shrinking, T_g sets the lower limit for thermal activation. Above T_g , stress relaxation and shrinkage can occur; when combined with a modulus gradient, this results in surface wrinkling. Therefore, precise control of thermal transitions is critical for processing and designing functional films. (2)

1.2.3 Young's Modulus and Thermomechanical Response

Young's modulus (E) quantifies the stiffness of a polymer and is defined as the slope of the initial, linear portion of the stress-strain curve. It is influenced by chain rigidity, degree of crystallinity, crosslink density, and temperature. Glassy polymers (below T_g) display high E due to restricted chain mobility, whereas rubbery polymers (above T_g) exhibit low E and high extensibility. (2)

For film-forming applications and wrinkling via thermal shrinking, polymers with a high E at room temperature (glassy state) and a sharp transition in modulus around T_g are preferred. This contrast in stiffness enables surface instability patterns like wrinkling to form during thermal treatment. By depositing stiff nanoparticle layers on a compliant substrate or vice versa, wrinkling wavelengths and amplitude can be tuned through the mismatch in modulus.

(2)

1.2.4 Relevance

Understanding the interplay between polymer structure and mechanical/thermal behaviour is essential for tailoring material performance. In the context of this thesis, where polymers are used to form thin films that wrinkle upon thermal treatment, aspects like Young's modulus, T_g , chain rigidity, and intermolecular interactions are directly relevant. Nanoparticles can further modify these properties, enhancing stiffness, thermal resistance, or introducing surface functionalities that tune the wrinkle morphology.

In subsequent sections, the specific polymers studied (e.g. polystyrene) and nanomaterials (e.g. polydopamine) will be discussed in more detail, focusing on how these fundamental polymer properties translate into the observed structural and functional outcomes in hierarchically structured surfaces.

1.3 Polystyrene

As previously mentioned, one of the most widely used synthetic polymers is polystyrene, primarily due to its well-balanced material properties, ease of processing, and environmentally safe production. It is essentially one of the major thermoplastics. Commercially, polystyrene is most commonly available in four main forms: general purpose polystyrene (GPPS), high impact polystyrene (HIPS)—also referred to as impact polystyrene (IPS), expanded polystyrene (EPS), and syndiotactic polystyrene (sPS), produced using metallocene catalysis. Each form serves specific functions depending on its mechanical properties, clarity, or thermal behaviour, making polystyrene an extremely versatile material across packaging, electronics, construction, and biomedical applications.

(9)

1.3.1 Styrene, the monomer

The monomer of polystyrene is styrene, also called vinylbenzene or ethenylbenzene. (10) Styrene was first isolated in 1831 from a balsam called storax, the resin from a genus of tree *Liquidambar*. (9–11) It is a sweet-smelling, colourless to yellowish liquid present naturally in some plants and in some foods due to microbial activity. Styrene is volatile and can also be traced in the air as a result of industrial activities. However, it is quickly removed through reactions with other atmospheric components. It is not miscible with water and degrades quickly in nature with a half-life of 15 days. Its biodegradation in soil is somewhat larger, with a half-life of 30 days, 300 days if deposited in sediment. Styrene has a relatively low acute toxicity; the oral median lethal dose (LD_{50}) of styrene in rats is approximately 5000 mg/kg. The median lethal concentration (LC_{50}) for a 4-hour inhalation exposure in rats is 2770 ppm. (10) Considering long-term exposure, the International Agency for Research on Cancer considers styrene to be probably carcinogenic to humans (group 2A). (10, 11)

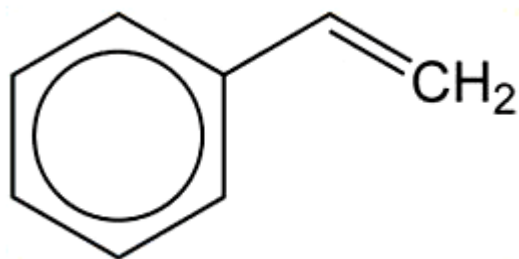


Figure 4 Styrene formula (12)

Styrene is industrially synthesized by first reacting benzene with ethylene to form ethylbenzene, which is subsequently dehydrogenated in a separate step. Vaughan et al. (2015) confirmed that another route to make styrene in only one step is possible by employing rhodium catalyst and recyclable copper salt as an oxidant to couple ethylene and benzene, although the suggested process seems to be too slow to be industrially applicable. (13)

1.3.2 Polymerization and production process of polystyrene

Styrene is prone to spontaneous polymerization when exposed to atmospheric oxygen and light. (10, 11) For controlled synthesis, it can be polymerized through various mechanisms, including radical, anionic, cationic, and metallocene-catalyzed polymerization. In industrial practice, polystyrene is primarily produced using bulk, solution, emulsion, or suspension polymerization techniques. (9) Among these, radical polymerization, particularly in bulk and suspension systems, is the most widely employed. (14)

In the production of thermoplastics, various additives are employed to enhance the durability and functionality of the material. These include antioxidants, flame retardants, processing lubricants, and stabilizers – each serving a specific function to improve the overall quality and lifespan of the polymer resin. (15) In addition to these traditional additives, recent developments have introduced a range of innovative materials, such as silver nanoparticles. Silver nanoparticles are utilized as antimicrobial agents in plastic food packaging, thereby extending the shelf life of food products. They achieve this by targeting microbial cells, disrupting their membranes, and inhibiting the enzymes responsible for nutrient transport, effectively preventing bacterial growth. Other additives, such as dyes and pigments, are incorporated to improve the aesthetic properties of plastics. (15, 16)

1.3.3 Properties of polystyrene

Commercial polystyrene (PS) is a non-crystalline polymer typically characterized by a weight-average molecular weight ranging from approximately 100,000 to 400,000 g/mol. PS is chemically stable in a wide range of pH from acidic to basic and also resistant to most salts, but can be attacked and dissolved by some organic solvents, e.g. acetone, benzene or tetrahydrofuran. (15)

At temperatures well below its glass transition temperature and under small mechanical loads, PS exhibits linear elastic behaviour in accordance with Hooke's law. Its mechanical strength – particularly in tensile and flexural modes – increases with molecular weight, but this effect levels off beyond a certain threshold. If the molecular weight falls below about 70,000 g/mol, the material becomes prone to failure and displays brittle behaviour. When subjected to high strain rates, PS develops microscopic defects known as crazes, which originate from voids connected by aligned fibrils and help dissipate impact energy across a wider area. Around the glass transition region, polystyrene shows viscoelastic melt behaviour, which depends primarily on its molecular weight and how evenly that weight is distributed among the chains. In polymers with narrow molecular weight distributions, the zero-shear viscosity tends to scale with approximately the third power of molecular weight, highlighting the sensitivity to chain length. Although the breadth of the molecular weight distribution has little effect on zero-shear viscosity itself, it plays a significant role in determining melt compliance and flow behaviour. Incorporating small amounts of high-molecular-weight polystyrene into a lower-Mw matrix can lead to improvements in elasticity and processing strength. (9)

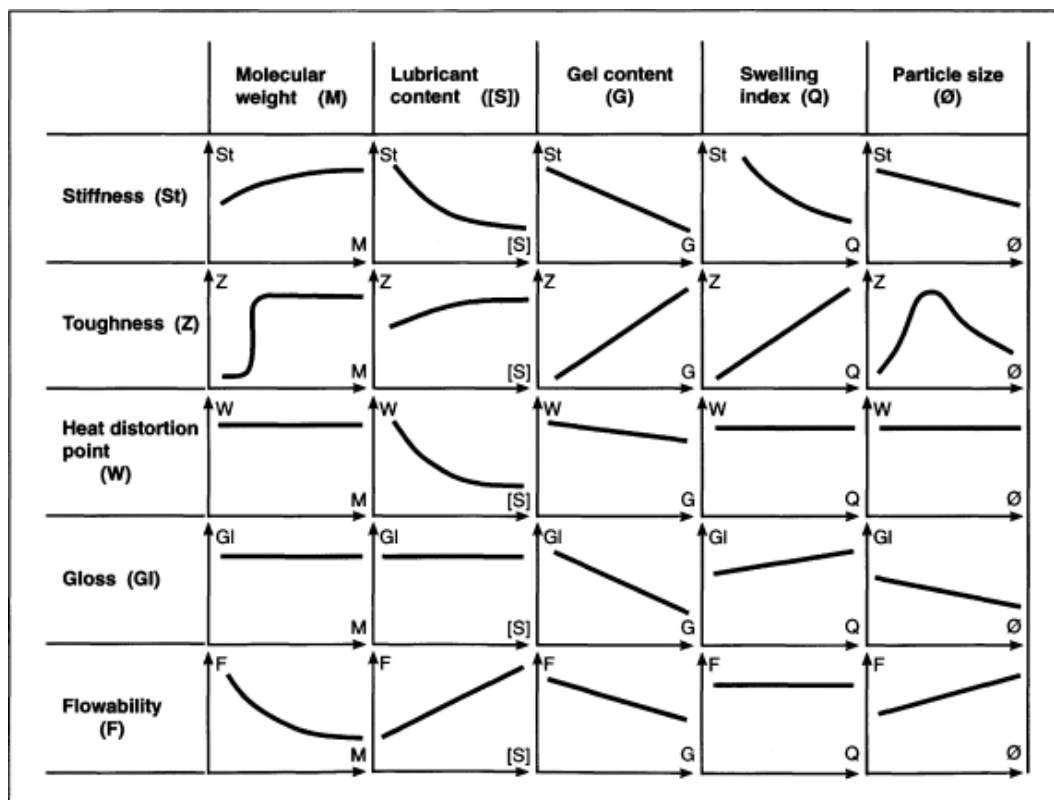


Figure 5 Schematic representation of the interrelationships between structure and properties of polystyrene (9)

Table 1 Properties of atactic PS(9)

Property	Unit	Test method	Value (GPPS) ^a	Value (IPS) ^b
Density	g cm ⁻³	ISO 1183	1.050	1.050
Refraction index	—	ISO 489	1.590	—
Coefficient of expansion	—	—	—	—
< T_g	cm ³ cm ⁻³ K ⁻¹	—	2.805×10^{-4}	—
> T_g	cm ³ cm ⁻³ K ⁻¹	—	5.650×10^{-4}	—
Glass transition temperature	°C	—	100	—
Heat deflection temp. under 1.8 MPa load (HDT A)	°C	ISO 75-2	86	73–85
Heat deflection temp. under 0.45 MPa load (HDT B)	°C	ISO 75-2	98	81–96
Vicat softening point A50	°C	ISO 306	101	91–101
Thermal conductivity	W (m K) ⁻¹	DIN 52 612	0.17	0.17
Dielectric constant at 100 Hz/1 MHz	—	IEC 60 250	2.5/2.5	2.5/2.5
Dissipation factor at 100 Hz/1 MHz	—	IEC 60 250	$0.9 \times 10^{-4} / 0.7 \times 10^{-4}$	$1.0 \times 10^{-4} / 4 \times 10^{-4}$
Volume resistivity	Ω m	IEC 6093	> 10 ¹⁴	> 10 ¹⁴
Tensile modulus of elasticity	MPa	ISO 527-2	3300	1400–2800
Tensile stress at yield	MPa	ISO 527-2	42–59	21–43
Elongation at yield	%	ISO 527-2	1.5–3.0	1.4–2.0
Charpy impact strength	—	—	—	—
at +23 °C	KJ m ⁻²	ISO 179/1eU	10–28	70–N.B.
at -30 °C	KJ m ⁻²	ISO 179/1eU	2–4	50–160

a GPPS: General purpose polystyrene. b IPS: Impact polystyrene. N.B.: No break.

PS is widely used due to its low density, optical clarity, surface gloss, dimensional stability, and ease of processing. (9)

1.3.4 Applications and modifications of polystyrene

Polystyrene's relative inertness enables its use in the biomedical field. Together with low-cost and easy fabrication and high optical clarity, PS replaced glass in tissue culture over 50 years ago. For tissue culture of adherent cells, which is most, some surface modification is necessary. Plasma (corona) treatment is used to enhance its wettability, providing better cell attachment. (17, 18)

Overall, there are several techniques for modifying polystyrene (PS) surfaces to enhance their functionality, particularly for biomedical applications like tissue engineering. Surface-initiated polymerization, including anionic and cationic methods, enables the grafting of functional polymer brushes onto PS, offering precise control over chemical functionality and surface properties. These modified surfaces can enhance biocompatibility and specific biomolecule interactions, crucial for tissue culture environments. (15)

Additionally, sulfonation of PS is used to introduce sulfonic acid groups, transforming PS into ion-exchange materials or flocculants, which can support cell attachment or purification processes in biomedical setups. Functionalization using polystyrene-hemin dots also enables catalytic chemiluminescence, supporting bioimaging and diagnostics with enhanced stability and biocompatibility – qualities that are promising for dynamic monitoring in tissue engineering contexts. (15)

PS is also a foundational material for electrospun nanofibers, which are used in scaffolds for tissue regeneration. Modified nanofibers – produced by blending with cellulose whiskers or chemical crosslinking – provide structural mimicry of extracellular matrices and allow functional molecule incorporation, offering mechanical strength and bioactivity. (15)

1.3.5 Pre-stressed polystyrene

Another form of PS that is also of interest for surface engineering and tissue engineering applications is pre-stressed polystyrene (psPS). (19) It is commercially and industrially used as shrink foil sold in stationery for crafting or as a shrink sleeve labelling material, respectively. (20, 21) This material is manufactured to store intrinsic tension, which is released upon heating above its glass transition temperature due to relaxation of the polymer chains. The ability to shrink, combined with a relatively low T_g , makes psPS an ideal material for surface structuring via thermal treatment, enabling the formation of wrinkles in adhered biopolymeric layers, as discussed in the chapter on polymer surface modification

methods. (22) Pre-stressed polystyrene is a type of general-purpose polystyrene (GPPS), which is atactic and therefore exhibits the typical behaviour listed in the table above. (20)

1.3.6 Polystyrene and environment

Plastic pollution represents one of the major environmental challenges of the modern era. Polystyrene is characterized by its high durability and resistance to degradation. Despite the implementation of various disposal methods, PS continues to pose risks to biodiversity, human health, and ecological systems. (23)

Even though PS undergoes biological decomposition – which, in natural environments, is extremely slow. (24, 25) Studies have shown that in cultivation soils containing various fungi, microbes, and invertebrates, the degradation of PS after 90 days was less than 1%, and after this period, no significant increase in the degradation rate was observed. (24)

The resistance of most thermoplastics to hydrolysis is largely due to their hydrophobic nature. (26, 27) The molecular structure of polymers influences their surface hydrophobicity, which in turn affects the potential adhesion of microorganisms. (25, 26, 28) Due to their high molecular weights and insolubility in water, microorganisms are unable to transport these plastics into their cells for metabolic purposes. (24, 25, 28)

Biological degradation can begin outside the microbial cell through the secretion of extracellular enzymes. (29, 30) However, these enzymes are too large to penetrate deep into the polymer, so they act on the surface by breaking down polymer chains via hydrolysis. (27, 29, 30) This process can be further facilitated by the formation of functional groups within the polymer chain. (26, 27)

1.4 Hyaluronic acid

A representative of natural polymers with many applications and potential in industry, medicine and research is hyaluronic acid. Hyaluronic acid (HA), also known as hyaluronan or sodium hyaluronate (NaHy), is a glycosaminoglycan belonging to the group of polysaccharides. (31)

1.4.1 Structural unit and occurrence

It is composed of alternating disaccharide units of β -(1,3)-N-acetylglucosamine and β -(1,4)-glucuronic acid, linked through β -1,4 and β -1,3 glycosidic bonds (see Figure 6.) Although the commonly used name includes the term "acid," NaHy is fully dissociated and negatively

charged under normal physiological conditions, typically existing in its anionic form in living organisms. Therefore, Balazs et al. proposed the term "hyaluronan" be used regardless of whether the molecule is in its acidic or dissociated state. (31)

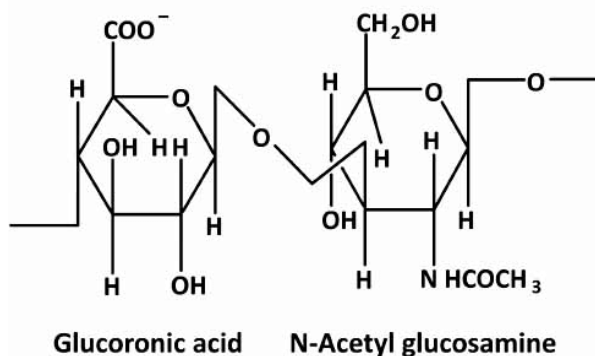


Figure 6 Structural unit of the hyaluronic acid chain (32)

Based on the number of repeating units, hyaluronan can be categorized into three main types: low molecular weight NaHy (6–200 kDa), medium molecular weight NaHy (0.2–1.0 MDa), and high molecular weight NaHy (≥ 1 MDa). In general, a hyaluronan chain can contain more than 10,000 repeating units, corresponding to a molecular weight of approximately 4 million Da. The hyaluronan molecule is energetically stable only when its sugar units adopt the so-called β -conformation, in which the anomeric carbon, hydroxyl group, and carboxyl group occupy sterically favorable equatorial positions, while hydrogen atoms are located in the less favorable axial positions. (33)

In solution, hyaluronan can adopt not only a linear primary structure, defined by the sequence of disaccharide units, but also secondary and tertiary structures. The secondary structure is characterized by a helical form that arises due to the 180° rotation of each successive disaccharide unit relative to the previous one. This structure is stabilized by hydrogen bonds formed between the carboxyl and acetylamino groups of HA and water molecules. Linear, unbranched NaHy chains can associate into a twisted structure known as a simple helix. When two such simple helices align antiparallel to one another, a double helix may form, representing the tertiary structure of hyaluronan. This arrangement is considered analogous to the β -sheet structure commonly found in proteins. (34–37)

The presence of carboxyl groups within the hyaluronan chain imparts a negative charge to the NaHy molecule at physiological pH, making it highly hydrophilic and capable of binding a substantial number of water molecules. This results in high viscosity, which is attributed to intra- and intermolecular hydrogen bonding. As previously noted, hyaluronan

can adopt complex spatial conformations depending on its surrounding environment. Spagnoli et al. demonstrated that the structure of HA adsorbed onto different surfaces may differ significantly from that of HA in tissues, likely due to its interactions with cells or proteins. (38)

1.4.2 Properties

Hyaluronan (HA) exhibits distinct physicochemical and biological characteristics that are closely linked to its molecular weight. It is typically classified into four molecular weight ranges: low (10–250 kDa), medium (250–1000 kDa), high (>1000 kDa), and very high (>6000 kDa). Except for the ultra-high molecular weight form, which is not found in humans, all others are naturally present in various tissues and contribute to physiological and pathological processes. (39–41)

The biological activity of HA is strongly influenced by its molecular size. Oligomeric HA (o-HA), typically under 10 kDa, and low and medium molecular weight forms can activate cellular responses such as inflammation, angiogenesis, and immune stimulation. These effects are mediated by size-dependent receptor binding and intracellular signaling pathways, which may involve heat shock proteins. These smaller fragments promote endothelial cell proliferation, migration, and tissue repair, playing a key role in processes such as wound healing and regeneration. (42, 43)

In contrast, high molecular weight HA is known for its anti-inflammatory and immunosuppressive effects. It contributes to extracellular matrix integrity and exhibits anti-angiogenic properties, helping regulate vascular responses. Its pronounced viscoelasticity and mechanical strength make it particularly suitable for biomedical applications requiring structural support, such as joint supplements and wound dressings. As a key component of synovial fluid, it also ensures proper joint lubrication and is widely used in osteoarthritis treatment. (44, 45)

Moreover, the ability of HA to permeate the skin also depends on its molecular weight. A study by Essendoubi et al. (2016) demonstrated that low molecular weight HA (20–300 kDa) is capable of penetrating into the deeper layers of the epidermis, while high molecular weight HA (1000–1400 kDa) largely remains confined to the stratum corneum. This distinction is critical in topical applications where depth of skin penetration determines therapeutic efficacy. Raman spectroscopy, a non-destructive and chemically specific technique, has proven valuable for assessing such permeation profiles. (46)

In the human body, hyaluronan is ubiquitously distributed across a wide range of tissues, with the highest concentrations found in the skin, where approximately 50% of the body's total HA is localized. It is also abundant in soft connective tissues such as the umbilical cord, synovial fluid, intervertebral discs, and the vitreous humor of the eye. Lower levels occur in organs such as the muscles, kidneys, and brain, while the lowest concentrations are typically found in blood serum. The total HA content in an average adult weighing 70 kg is estimated to be around 15 grams, the majority of which is situated in the extracellular matrix and skin. Despite its relatively large total mass, HA is subject to rapid turnover, with approximately one-third of the total pool (about 5 grams) being synthesized and degraded daily. (47, 48) The synthesis of hyaluronan occurs within cells through the action of specific enzymes, after which the polymer is extruded across the plasma membrane into the extracellular matrix. Among the principal cell types involved in its production are fibroblasts, chondrocytes, and keratinocytes, which are responsible for maintaining HA levels in connective tissues, cartilage, and the skin, respectively. (49)

Most of the hyaluronan synthesized in the human body exists as high molecular weight polymers, typically around 2000 kDa, with HAS2 being the primary enzyme responsible for their production. Other molecular weight forms are generated secondarily through enzymatic or oxidative degradation of these high molecular weight chains. This degradation is predominantly catalyzed by hyaluronidases, particularly HYAL1 and HYAL2, which are widely expressed in somatic tissues. These enzymes sequentially break down HA into smaller fragments, contributing to its rapid turnover and functional diversification. (37, 50) Historically, hyaluronan was first isolated by Meyer and Palmer in 1934 from bovine vitreous humor. Since then, it has also been successfully extracted from a variety of other animal-derived tissues, including the umbilical cord, rooster comb, and synovial fluid. In addition to these traditional sources, modern biotechnological methods have enabled the production of HA using microbial fermentation processes, further broadening its availability for biomedical and industrial applications. (37, 51, 52)

While animal-derived tissues have historically served as a primary source of hyaluronan, their use presents several limitations. The extraction process is typically multi-stage and labour-intensive, often resulting in low yields that increase production costs and constrain availability. Moreover, residual host-derived proteins and nucleic acids present in the final product may provoke immune or allergic reactions, raising concerns about product safety. Another critical factor is the natural presence of hyaluronidases in animal tissues—enzymes

capable of degrading HA prior to its isolation, leading to reduced molecular weight, lower recovery rates, and compromised purity of the final extract. (49, 53)

In response to the limitations associated with HA extraction from animal tissues – including low yield, risk of immune responses, and enzymatic degradation – alternative biosynthetic approaches have been investigated. Among these, certain bacteria such as *Pasteurella multocida* and Gram-positive *Streptococcus* species from groups A and C have attracted attention due to their intrinsic ability to synthesize and secrete HA as a secondary metabolite. While these microorganisms offer a promising route for large-scale production, their application in biomedical fields remains constrained by the presence of bacterial endotoxins, which necessitates extensive downstream purification. (49)

Today, industrial-scale hyaluronan production relies primarily on two approaches: extraction from animal tissues and microbial fermentation using specific bacterial strains. Both processes yield polydisperse HA with molecular weights typically exceeding 1,000 kDa and a polydispersity index ranging from 1.2 to 2.3. Microbial fermentation, first implemented in the 1960s, offers a relatively efficient and controllable production route. A key advantage of this method is the ability to physiologically or metabolically modulate microbial cultures to favor the synthesis of high molecular weight HA, enhancing its suitability for biomedical and cosmetic applications. (53) Some of the leading producers of sodium hyaluronate (HA-Na) include companies such as Contipro a.s., Alfa Sagittarius, and HTL Biotechnology. (54)

The physicochemical characteristics of hyaluronic acid (HA) are fundamental to its function within the extracellular matrix (ECM), particularly in maintaining tissue hydration. Due to its strong hygroscopic capacity, HA acts as an effective water-binding agent, thereby contributing to the mechanical resilience and moisture retention of connective tissues. This property is especially critical in the dermis and synovial fluid, where HA supports both structural integrity and physiological elasticity. (55)

1.4.3 Applications

The structural adaptability of hyaluronic acid enables the development of a wide range of material forms, including viscoelastic solutions, hydrogels, nanofibers, flexible membranes, and biodegradable carrier systems. These engineered formats significantly expand HA's utility across biomedical applications. In particular, carrier matrices constructed from hyaluronan function as temporary scaffolding materials that facilitate cell adhesion, proliferation, and tissue regeneration. Biodegradable HA-based structures such as hydrogels

are increasingly employed in clinical contexts, supporting applications in bone repair, spatial tissue filling, neural regeneration, cell transplantation, and skeletal muscle healing. Their transient nature ensures integration into host tissue without the need for surgical removal. (56)

Hydrogel-based formulations of hyaluronic acid have demonstrated promising effects in dermal applications by enhancing hydration and contributing to visible skin rejuvenation. Clinical evaluation of stabilized non-animal-derived HA gels showed significant improvements in skin elasticity, surface smoothness, and patient-reported outcomes. These effects are believed to arise from HA's intrinsic hygroscopic properties and its ability to modulate the extracellular matrix, including stimulation of Type I collagen production. Such findings highlight the potential of HA hydrogels not only to restore skin function but also to support tissue remodeling processes that may reduce fibrotic changes over time. (57)

In certain applications, hybrid materials combining hyaluronic acid-based hydrogels with micro- to nanoscale fibers have demonstrated enhanced performance. These composite systems leverage the high specific surface area, interconnected porosity, and fine pore architecture of nanofibers to closely replicate the structure of the native extracellular matrix. As a result, they provide a favorable microenvironment that promotes cell adhesion, migration, and proliferation—key processes in effective wound healing and tissue regeneration. Additionally, HA nanofibers are particularly attractive for drug delivery due to their rapid hydration and dissolution upon exposure to moist conditions, enabling immediate release of encapsulated therapeutics. However, this rapid degradation profile renders them unsuitable for applications requiring long-term structural stability or sustained release. (58, 59)

Building upon the functionality of fiber-based systems, hyaluronic acid has also been incorporated into self-assembling nanoparticle formats, further expanding its utility in advanced biomedical technologies. Notably, hyaluronic acid nanoparticles (HA-NPs) have gained attention in oncology for their application in drug delivery. These particles typically consist of a hydrophobic core and a hydrophilic HA shell. Studies have shown that HA-NPs accumulate more effectively in tumor tissues compared to water-soluble HA derivatives, and their biodistribution is influenced by key physicochemical properties such as surface charge, particle size, and molecular weight. However, the precise role of these factors in governing in vivo therapeutic efficacy remains incompletely understood. (60, 61)

Beyond oncology, HA-NPs have also demonstrated utility in skin regeneration therapies, particularly in addressing UV-induced aging. Research indicates that UV exposure leads to a measurable decline in endogenous HA within the skin, in part because the large molecular size of native HA limits its ability to penetrate the epidermis. In contrast, nanoscale formulations allow for more effective transdermal delivery, facilitating high local concentrations of HA without the need for invasive methods such as injection. (62)

Together, these applications illustrate the remarkable versatility of hyaluronic acid in both regenerative medicine and targeted drug delivery, reinforcing its role as a foundational biomaterial in modern biomedical science.

2 NANOPARTICLES / NANOMATERIALS

Nanomaterials represent one of the fastest-growing fields in materials science. Examples include fullerenes, graphene flakes, and single-walled carbon nanotubes. Nanomaterials are defined as materials with at least one external dimension in the range of 1–100 nm. This nanoscale size imparts unique physical and chemical properties compared to their bulk counterparts. However, alongside their development, it is essential to consider their potential toxicity and ecotoxicity to assess risks to human health, society, and the environment. (63)

According to ISO/TS 27687:2008, a nanomaterial is any engineered product, substance, or structure with at least one dimension in the nanoscale. Additionally, materials that fall outside this range in size but exhibit nanoscale-specific properties, such as high surface area or altered optical and mechanical behaviour, are also classified as nanomaterials. This includes nanoparticles (three nanoscale dimensions), nanofibers (two nanoscale dimensions and one elongated axis), and nanoplates (one nanoscale dimension). (64)

Nanoparticles (NPs) can vary in shape (spherical, cylindrical, conical, tubular, hollow-core, helical, irregular), size (clusters if <1 nm), and structure (monocrystalline, polycrystalline, or amorphous). They may be homogeneous or composed of multiple layers: (a) a surface layer of small molecules, metal ions, surfactants, or polymers; (b) a shell of chemically distinct material; and (c) a core forming the NP's central component. (65)

Due to their small size and high surface area, nanoparticles often exhibit unique properties that are size-dependent. Nanomaterials are interesting from various points of view across and applied across a range of fields, including environmental science (e.g., waste remediation, environmental monitoring (66), biotechnology, biomedicine (e.g., antimicrobial agents (67), and pharmaceuticals. They are studied for their anti-inflammatory and antibacterial activity, drug delivery efficiency, bioactivity, bioavailability and absorption, and targeted action against tumors. (68)

Nanoparticles exhibit distinct properties compared to their bulk counterparts due to several fundamental differences: (1) a high surface area with a large number of particles per unit mass, (2) a greater proportion of atoms located at the surface, and (3) surface atoms that are coordinated with fewer neighboring atoms. (69) As a result, both the physical and chemical properties of nanomaterials are altered

Nanomaterials also display increased chemical reactivity due to their high specific surface area and elevated surface-to-volume ratio. These surface phenomena significantly

influence structural behavior. Strong attractive forces between nanoparticles can cause aggregation and agglomeration, which may negatively affect surface-related properties. Such aggregation can be mitigated by increasing the zeta potential (to enhance repulsive forces), optimizing hydrophilic/hydrophobic balance, or adjusting pH and ionic strength. (65)

Nanoparticles are generally classified according to various criteria, including their dimensionality, morphology, composition, uniformity, and degree of agglomeration. Based on dimensionality, nanomaterials can be divided into four main categories. Zero-dimensional (0D) nanomaterials have all dimensions confined within the nanoscale range and include structures such as quantum dots, fullerenes, and spherical nanoparticles. One-dimensional (1D) nanomaterials, such as nanotubes, nanofibers, nanorods, and nanohorns, possess one extended dimension beyond the nanoscale, while the remaining two dimensions remain within it. Two-dimensional (2D) nanomaterials are characterized by two extended dimensions with nanoscale thickness and include forms such as nanoparticles adhered to substrates, porous thin films, nanosheets, nanofilms, and nanolayers containing nanoscale pores. Finally, three-dimensional (3D) nanomaterials have all three dimensions within the nanoscale and are often organized on a substrate, as seen in nanoporous membranes or surface-anchored nanostructures. (102)

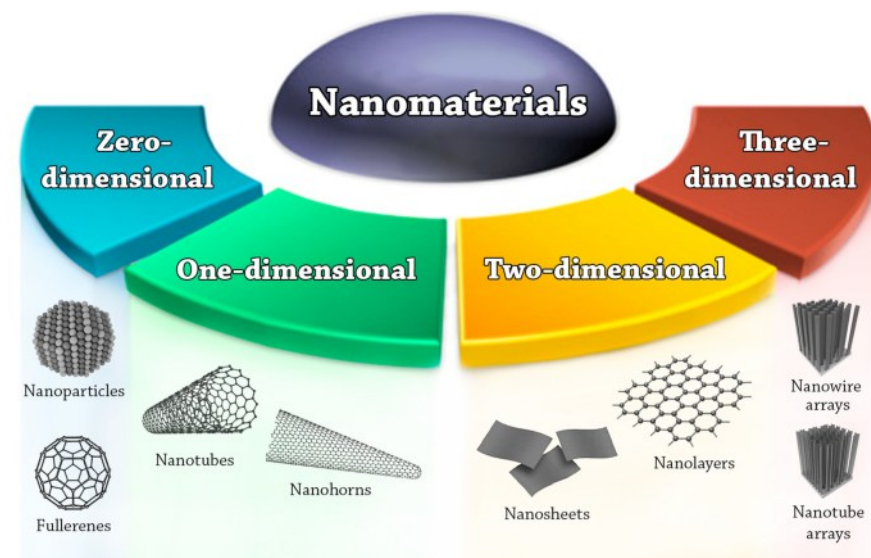


Figure 7 Nanomaterial classification (102)

In general, nanomaterials are synthesized using a variety of methods, which are categorized into two main categories: bottom-up and top-down methods (102), as shown in Figure 8.

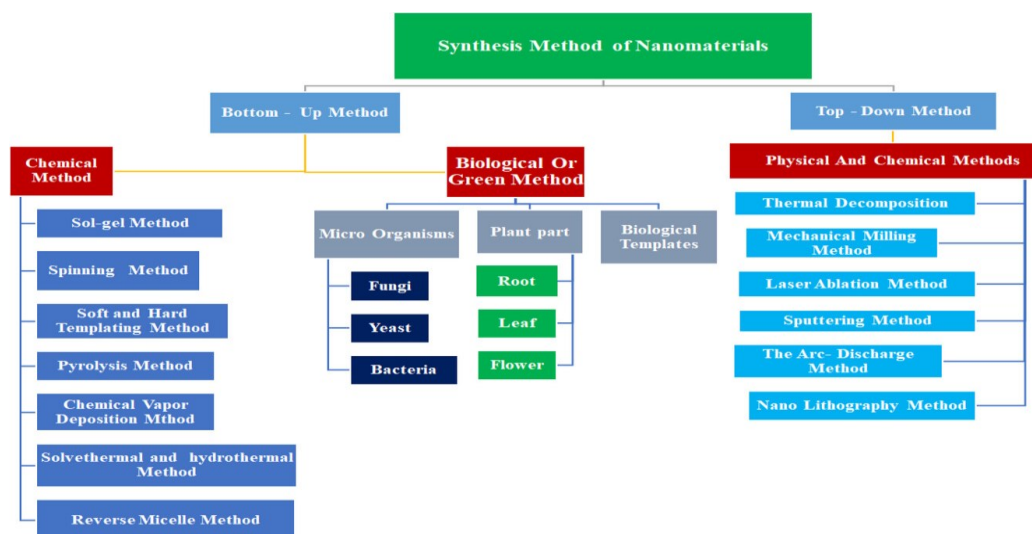


Figure 8 Different nanomaterial preparations (102)

2.1 Lysozyme nanofibers

Lysozyme is a well-studied and widely used protein with enzymatic activity that has recently been applied to prepare amyloid fibres via self-assembly, exhibiting distinct properties such as broad antimicrobial activity and intriguing mechanical characteristics.

2.1.1 Structure of native lysozyme

Lysozyme is a well-studied, relatively small enzyme composed of approximately 129 amino acids and characterized by a compact globular structure. (70) Lysozyme is also referred to as 1,4-beta-N-acetylmuramidase C. (71) Its primary sequence includes several cysteine residues forming four disulfide bridges that stabilize the overall conformation. In terms of secondary structure, lysozyme features a mix of α -helices and β -sheets; notably, its α -domain contains four helices, while the β -domain comprises a twisted antiparallel β -sheet. These structural elements contribute to a distinct cleft between the two domains where the catalytic activity occurs. The tertiary structure is further stabilized by internal hydrogen bonding and hydrophobic interactions, contributing to its thermal and chemical stability under physiological conditions. (70)

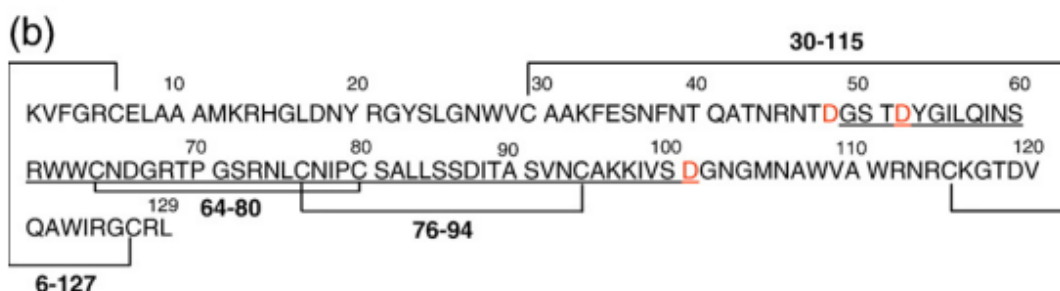


Figure 9 Primary structure of Hen Egg white Lysozyme (HEWL) (70)



Figure 10 Tertiary structure of chicken-derived lysozyme (71)

2.1.2 Functions of native lysozyme in nature and the human body

Lysozyme serves as an innate immune defense molecule. It cleaves the β -1,4 glycosidic bond between N-acetylglucosamine and N-acetylmuramic acid in peptidoglycans, effectively lysing bacterial cell walls. This enzymatic activity is especially prominent in bodily secretions such as saliva, tears, mucus, and also in leukocytes. (70)

2.1.3 Applications in Industry, Medicine, and Research

Due to its bacteriolytic properties, lysozyme is utilized in food preservation, especially in cheese and wine industries, to prevent spoilage. In pharmaceuticals, it serves as an anti-inflammatory and antiviral agent and has been explored for wound healing and gastrointestinal health. Hen egg-white lysozyme (HEWL) is often used in research as a model protein to study protein folding, aggregation, and amyloidosis due to its well-

characterized structure, ease of purification, and ability to form amyloid fibrils under specific conditions. (70)

2.1.4 Function and Impact of protein amyloids

Amyloids are highly ordered protein aggregates composed of β -sheet-rich fibrils exhibiting a characteristic cross- β structure. While often associated with pathological states such as Alzheimer's, Parkinson's, and systemic amyloidoses, amyloids are not exclusively harmful. In functional contexts, they play physiological roles in bacteria (e.g., biofilm formation), fungi (e.g., prion-based inheritance), and even humans (e.g., hormone storage in secretory granules). (72)

2.1.5 Amyloid fibril Formation Mechanism

Amyloid fibril formation generally follows a nucleation-dependent pathway. Initially, native or partially unfolded proteins undergo conformational changes to expose hydrophobic regions and β -sheet-prone sequences. This is followed by oligomerization, nucleation, and elongation phases, where monomers align into cross- β sheet structures stabilized by hydrogen bonding and steric zipper interactions. The transformation is energetically driven by a balance between enthalpic gains (from hydrogen bonds and van der Waals contacts) and entropic losses due to reduced molecular freedom. (72)

Various environmental factors, such as acidic pH, elevated temperature, and agitation, can promote amyloidogenesis. For lysozyme, acidic conditions (pH 1.6–2) and heat (typically 57–70 °C) facilitate partial unfolding and hydrolytic fragmentation, producing highly amyloidogenic fragments (notably residues 49–101 and 53–101) that nucleate fibril growth. (70)

2.1.6 Lysozyme Nanofibers (Amyloids)

Lysozyme nanofibers (LNF) are typically produced through a top-down process involving the partial hydrolysis and denaturation of full-length lysozyme. Under amyloidogenic conditions, HEWL (hen egg-white lysozyme) fragments into highly fibrillogenic peptides. The main fibril core consists of the 49–101 or 53–101 fragment, while non-amyloidogenic regions (1–48 and 102–129) are either excluded or contribute to steric hindrance that slows fibril formation. (70)

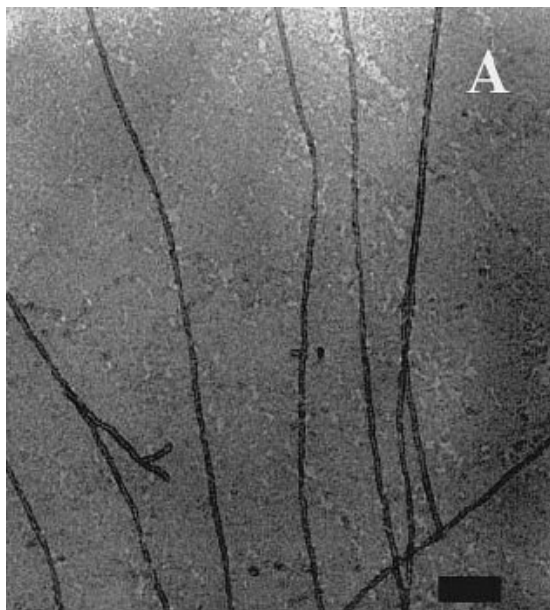


Figure 11 A representative TEM picture of lysozyme amyloid fibrils (73)

LNFs exhibit hallmark cross- β sheet signatures, including birefringence with Congo red and enhanced thioflavin T fluorescence. Electron microscopy reveals unbranched fibrils, typically 10–15 nm in diameter and up to several micrometers in length. These fibers are mechanically robust and thermodynamically stable due to dense hydrogen bonding and side-chain packing. (70)

Current and potential applications of LNFs span several domains:

In biomaterials - their biocompatibility and rigidity make LNFs promising scaffolds in tissue engineering. In drug delivery – LNF can encapsulate or bind therapeutic agents for controlled release. In biosensing – their ordered nanostructure allows functionalization with ligands or dyes for biosensor development. In fundamental studies, LNFs serve as a model system for understanding amyloid diseases and protein misfolding. Furthermore, the inherent immunogenicity and cytotoxicity of certain LNF forms have implications for both therapeutic risk assessment and vaccine adjuvant research. (70)

2.2 Polydopamine

Polydopamine (PDA) is a polymeric nanomaterial composed of dopamine (DA) monomers. Polydopamine was inspired by a naturally occurring polymer used by several mussel species to attach to practically any surface. As with many other natural phenomena, the ability of mussels to do this caught the attention of scientists because adhesion in wet environments can be challenging to achieve. Dopamine is analogous to the naturally occurring L-

dihydroxyphenylalanine (L-DOPA), which, along with amines such as lysine and histidine, is part of the mussel adhesive proteins. (74) In 2007, Le et al. demonstrated that dopamine is able to auto-polymerize and form thin adhesive films on a variety of surfaces (metals, oxides, ceramics, polymers). (75) Since then, polydopamine has been under investigation, mainly due to its adhesive properties and natural origin, with potential applications in material coatings, tissue engineering, biosensors, energy storage, environmental remediation, drug delivery etc. (74)

2.2.1 Dopamine

Dopamine, the monomer of polydopamine, is a natural substance found in the human body as a neurotransmitter and hormone. (76) Neurotransmitters are endogenous chemicals enabling communication between neurons through vesicle release from the presynaptic cell, uptake by receptors of pre- or post-synaptic cells, and rapid degradation or removal. (77) DA is considered to be one of the most important of more than a hundred known neurotransmitters. (78) Synthesised in parts of the brain known as the substantia nigra, ventral tegmental area and hypothalamus, it is part of the catecholamine pathway, as shown in Figure 12 starting from with the precursor phenylalanine, which enzymatically transforms into tyrosine and then into L-DOPA (79) (also present in the aforementioned mussel proteins). Decarboxylation of L-Dopa produces dopamine, which can then be converted into two other catecholamine neurotransmitters, norepinephrine or, even further, epinephrine. (80)

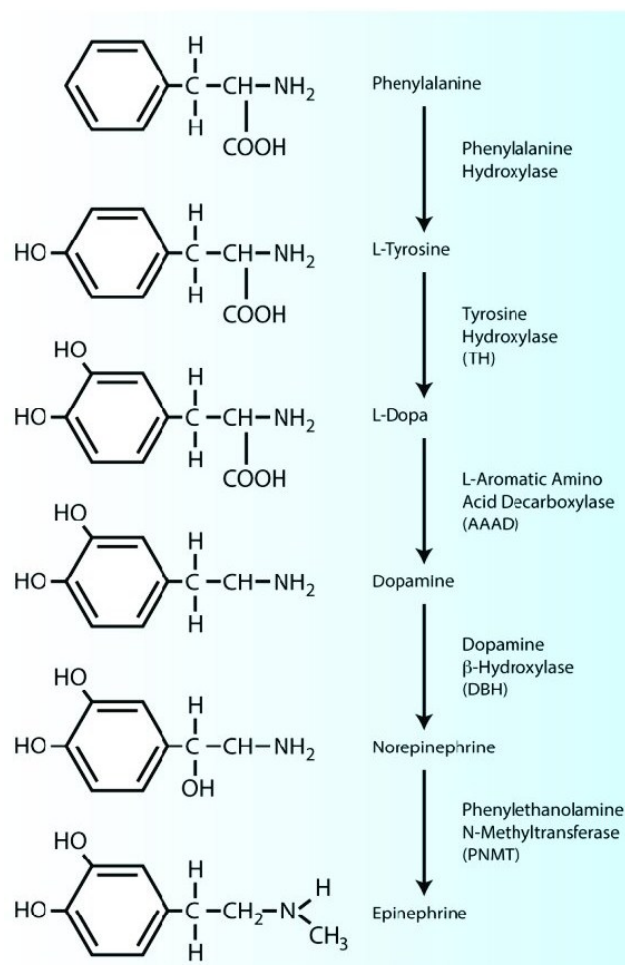


Figure 12 Catecholamine synthesis pathway (79)

Dopamine binds to five types of receptors referred to as D1 to D5, all of which are coupled to G-proteins and are predominantly expressed in the central nervous system. Although they are expressed in specific areas, some overlap. When DA binds to D1 or D5 receptors, there is a stimulatory effect on adenylyl cyclase activation, which further activates second messenger cAMP and leads to protein kinase A production, while binding to D2-D4 receptors has an inhibitory effect on adenylyl cyclase and leads to opening of potassium channels. (80) The D1 and D2 receptors are the most abundant, having the opposite effect. Dopamine influences the production of prolactin and is crucial for nearly all cognitive functions, including motor control, thought pathways, and the reward system, which is important for learning. (81, 82) On the other hand, it plays a significant role in the formation of addiction. Dysregulation of dopamine synthesis, release, and receptors has been implicated in several, if not all, neurological disorders, including depression, Parkinson's disease, and schizophrenia, to name a few. (82)

Another structurally analogous substance to polydopamine found in the human body is a dark-brown pigment able to absorb UV, melanin. This biopolymer consists of repeating units of phenolic and indolic compounds, as shown in Figure 13. (83)

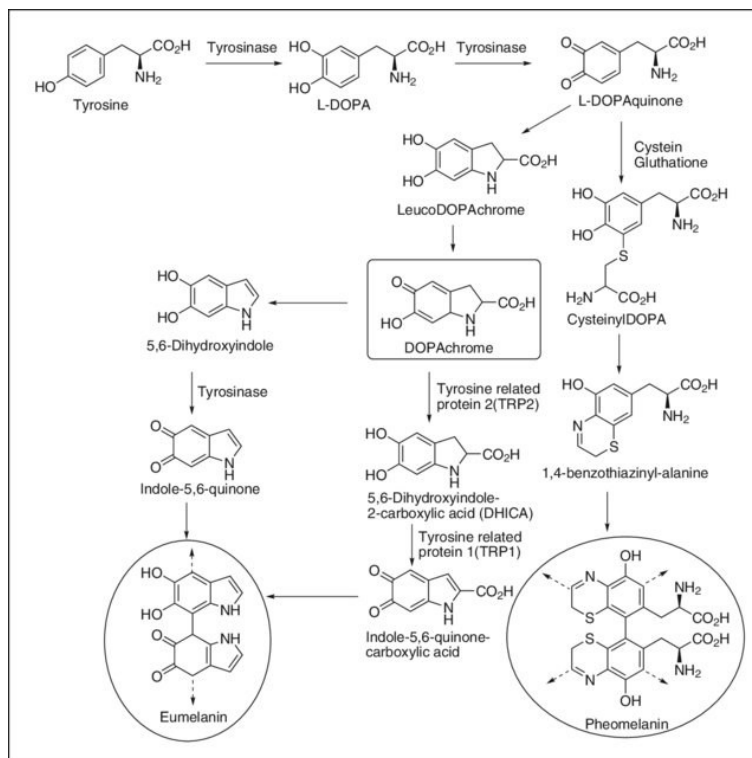


Figure 13 Melanin synthesis pathways (83)

2.2.2 Polymerization mechanism

The entire mechanism by which DA polymerizes into polydopamine is still not fully understood or described. However, many attempts have been made, and several models of this process have been proposed. They are summarized in Figure 14. Although the monomer DA is a relatively simple molecule, it undergoes several changes, resulting in a polymer of unclear structure composed of various building blocks with different degrees of oxidation, cyclization, and tautomerism. (74)

The first stage of the PDA synthesis is described in various publications. Initially, it involves an auto-oxidative reaction of dopamine to dopamine quinone (DAQ), followed by cyclization and isomerization. However, studies do not agree on the subsequent steps toward PDA formation. The models developed to describe the process can be divided into three categories, as reviewed by Hemmatpour et al. (2023) (74)

Four polymeric models have been presented: the so-called “Eumelanin model,” suggesting that PDA follows a similar pathway as melanin in organisms and that 5,6-

dihydroxyindole (DHI) is the main monomer of PDA. The “Open-chain poly(catechol/quinone) model” proposes that PDA is a linear chain of catecholamine monomers bonded via biphenyl-type bonds. Another polymeric model describes PDA as comprising three components – cyclized indole monomers, uncyclized catecholamines, and pyrrole dicarboxylic acid molecules. In this model, tris(hydroxymethyl)aminomethane (TRIS) units are covalently incorporated into PDA’s structure, as PDA is often prepared using a buffer such as TRIS. The last polymeric model views PDA as a chain similar to eumelanin, consisting of dopamine and DHI units of different degree of saturation and oxidation. (74)

Although there is evidence for the high molecular weight of PDA claiming the units are covalently bound, there are also studies claiming that PDA monomers are held together by physical forces such as Van der Waals interactions, hydrogen bonding, π - π interactions, charge transfer interactions. The chemical identity of the repeating units and their position toward each other are an object of discussion with no clear answers and reliable evidence, but a variety of models have been suggested. (74)

The third category of models describing PDA molecular structure can be referred to as trimer-based. A lot of studies have argued that PDA is mainly formed by aggregates of oligomers, especially trimers, which are formed by coupling oxidation and subsequently attracted by non-covalent interactions. (74)

Some of the methods used to investigate the character of PDA structure included mass spectroscopy, nuclear magnetic resonance (NMR), high-performance liquid chromatography and isotope-labelling techniques. Although broadly used, mass spectroscopy does not seem to be an adequate technique to distinguish between species of similar molecular weight but different functional groups. Thus, the conclusions of many studies are unreliable. (74)

In their study, Hemmatpour et al. aimed to resolve the longstanding ambiguities surrounding the mechanism of PDA formation by investigating the polymerization process on halloysite nanotubes (HNTs), which provide a high surface area. The HNTs’ outer surface is composed of negatively charged walls, while the inner surface has a positive charge, which enables an efficient way to capture reaction intermediates. This approach slowed the rapid kinetics of PDA polymerization, allowing a detailed analysis of structural evolution. Through spectroscopic techniques including X-ray photoelectron spectroscopy (XPS) and

solid-state ^{13}C NMR, they traced the sequence of molecular transformations from dopamine monomers to complex PDA networks. (74)

A key discovery was the identification of polycatecholamine as an early intermediate formed via oxidative coupling between dopamine and dopamine-quinone, rather than through pathways dominated by 5,6-dihydroxyindole, which many prior models emphasized. Their data show that cyclization into dopaminechrome (DAC), subsequent tautomerization, and partial oxidative cleavage into pyrroline dicarboxylic acid (PDCA) follow in later stages. Notably, TRIS buffer molecules were found to be incorporated into the initial PDA structure, a factor often overlooked in previous models. These intermediates aggregate via π - π stacking, H-bonding, and covalent bonding, forming the final cross-linked supramolecular network of PDA. (74)

This work advanced the understanding of PDA film formation, showing that the dominant process involves covalent polycatecholamine intermediates and not simply DHI-based units or non-covalent assemblies. The proposed stepwise model correlates closely with melanin biosynthesis and clarifies discrepancies among earlier structural hypotheses. (74)

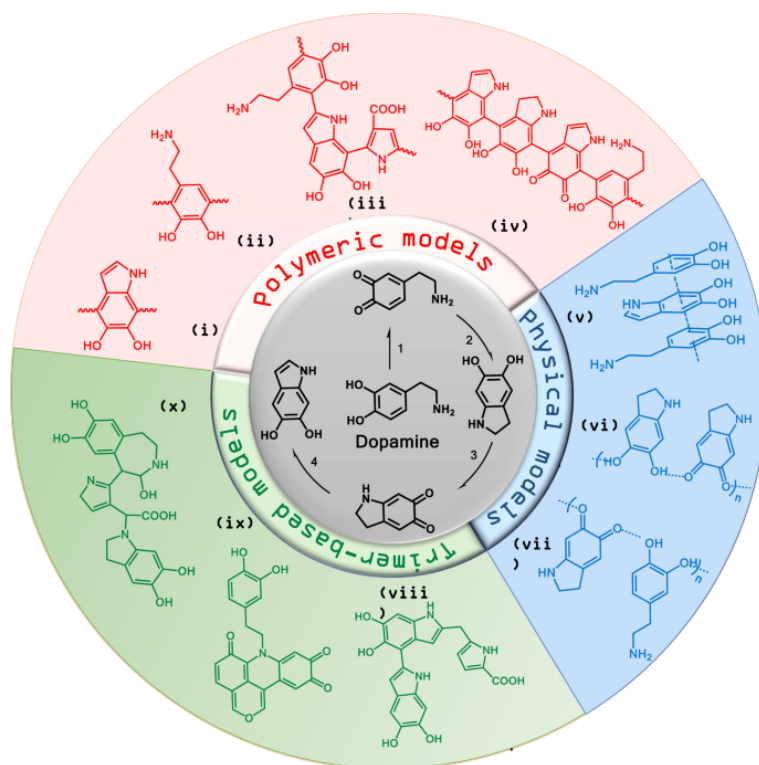


Figure 14 Polydopamine structural models (74)

2.2.3 Polydopamine synthesis

PDA deposition and polymerization occur simultaneously. The most common and straightforward protocol for PDA coating involves immersing a surface in an aqueous dopamine solution with a basic pH, allowing DA to polymerize and adhere to the surface, forming a film. At the same time, particles are synthesized in the solution and consequently adhere to the surface. Typically, pH is controlled by a TRIS buffer valued at 8.5, and atmospheric oxygen acts as an oxidant. (74)

However, several variables have been investigated, including pH values, buffers, additional solvents such as alcohols and oxidizing agents, as well as DA concentrations, substrates, and immersion times. (84)

2.2.4 Properties and applications

Polydopamine gained scientific interest across a broad variety of fields, mainly due to its outstanding and universal adhesiveness. Moreover, it has been proven by several studies that PDA is biocompatible (LD50 is 483.95 mg/kg), has a good antioxidant ability, and has high photothermal efficiency. All of these properties make PDA a great material for coating or as an intermediate layer facilitating the loading of another layer with different functionality without the necessity to alter the intrinsic properties of the substrate. (85)

The ability of PDA to bind to almost any organic or inorganic surface comes from its molecular structure as it comprises abundant catechol groups, amines and imines. (85)

Polydopamine has been prepared in various forms and shapes, such as film coatings, spherical nanoparticles (NPs), rods, nanocapsules, mesoporous NPs etc. (85)

PDA found application in regenerative medicine, where it has improved mechanical properties of scaffolds, but also served as a coating to improve cell attachment, differentiation and proliferation, with the ability to dampen inflammation through free radical scavenging. It appears that PDA promotes the attachment of various types of cells and tissues and has been investigated for various applications in the human body; some examples are given in a timeline in Figure 15. When applied to wounds or tumours, PDA can transform radiation into heat, aiding the reduction of cancer or bacteria in wounds, leading to faster recovery. (85)

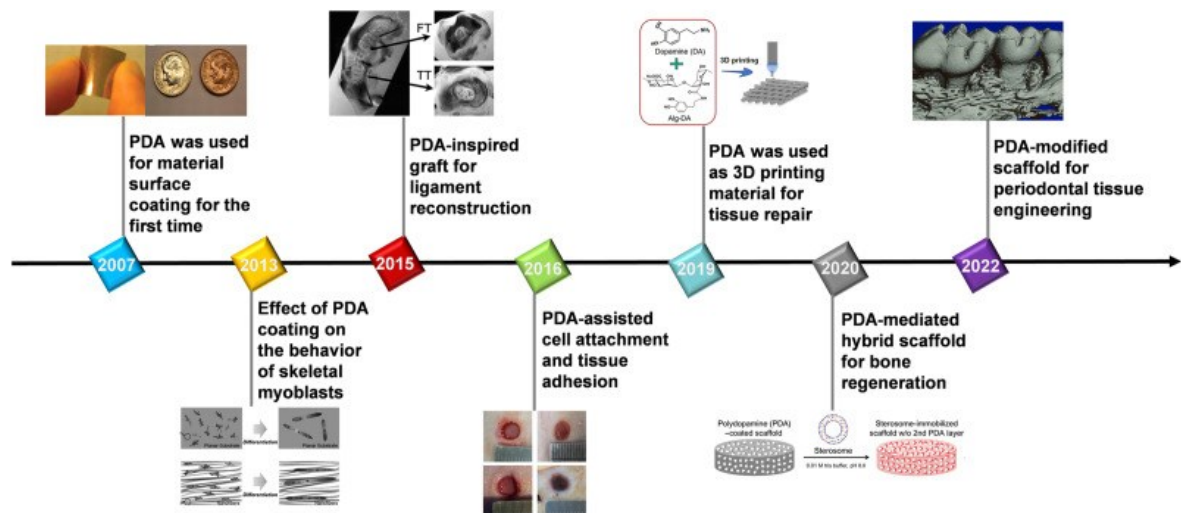


Figure 15 A brief timeline of the application of PDA in regenerative medicine (85)

Other interesting properties of PDA include metal chelation and electrical conductivity when combined with other materials such as gold. Recent advancements have significantly expanded the utility of polydopamine (PDA) by overcoming its longstanding limitation of poor electrical conductivity. Traditionally, PDA is usually considered to exhibit semi-conductive characteristics, having the conductivity around 10^{-5} – 10^{-13} S/cm, depending on its hydration state. Kim et al. (2022) developed a direct electrochemical synthesis method to produce highly conductive PDA coatings on gold (Au) electrodes. By exploiting the confined oxidizing effect of in situ generated Au^{3+} ions without the addition of chemical oxidants or buffering agents, they achieved site-specific and molecularly aligned PDA deposition. This approach resulted in PDA/Au composites with a conductivity as high as 1.17 S/cm, several orders of magnitude higher than that of conventional PDA. The conductive PDA coatings significantly improved electrochemical performance. The development of highly conductive PDA greatly broadens its potential applications, making it suitable for implantable and biodegradable electrodes, biosensors, neuromorphic electrochemical devices, and other bioelectronic systems that require both biocompatibility and efficient charge transfer. (86)

3 METHODS FOR POLYMER SURFACE MODIFICATION

In many materials, the surface characteristics differ from the bulk properties due to the distinct molecular environment and energetic state at the interface. (87, 88) In this context, surfaces typically represent an interface between a solid and a liquid or a gaseous medium. While the bulk is governed by the overall structure and composition, the surface is shaped primarily by the outermost atomic layers, making it highly sensitive to contamination, segregation, and environmental exposure. This contrast means that even slight surface alterations can profoundly impact properties like adhesion, wettability, optical appearance, and biocompatibility. (88) Surface energy reflects the energetic state of the outermost molecular layers and plays a critical role in phenomena like adhesion and film formation. Wettability describes how easily a liquid spreads across a surface and is influenced by the balance of surface energies between solid/liquid phases. (89) Closely related, the contact angle provides a measurable indicator of surface hydrophilicity or hydrophobicity. While surface tension is a property traditionally associated with liquids, it is similarly used to characterize the interfacial behavior of solids. The chemical composition of the surface determines its reactivity, functionalization potential, and compatibility with other materials. Molecular orientation can differ at the interface compared to the bulk, impacting polarity and crystallinity. Topography and roughness, including nano- and microscale structures, influence surface interactions such as friction, light reflection, and biological responses. (88)

It is often crucial to maintain the inherent properties of the material, e.g. its mechanical properties and, while using it in different environments where different surface properties are required, such as antifouling properties of membranes for water treatment or cell attachment of implanted biomaterials. (90, 91) Therefore, a distinct subtype of material engineering, referred to as surface engineering, emerged. Surface engineering investigates ways to alter surface topography, functionality, or other properties on demand of specific industrial or research needs. (87, 92) Surface modification can be defined as a process through which the physical, chemical or biological behaviour of a material is changed without majorly affecting its bulk properties. (87)

Some of the significant applications of modified surfaces include wear and corrosion-resistant coatings, improving biocompatibility of materials used in medicine, tailoring surface energy and wettability to promote better adhesion or, on the contrary, create an anti-fouling effect. Furthermore, enhancing conductivity or heat transfer is desirable in some applications. (87)

The properties of the surface can be altered by methods that directly attack the material, changing its superficial layers, or by adding an additional thin layer (film) to coat the material. The two following sub-chapters describe these two approaches separately.

3.1 Material surface modification methods

Polymer surface modification techniques can be broadly categorized into physical, chemical, thermal, plasma-based and special patterning methods. These approaches aim to adjust properties like surface energy, roughness, adhesion, or wettability without compromising the polymer's bulk characteristics. (93)

Physical methods include techniques such as extrusion, lamination, and imprinting to introduce micro- or nanoscale roughness. These are widely used for creating superhydrophobic or abrasion-resistant surfaces but do not alter the chemical nature of the material. (93)

Plasma treatments utilize ionized gases (e.g., O₂, Ar) to clean, activate, or functionalize the polymer surface. This method enhances surface energy and wettability by introducing polar groups and is especially effective for fragile or chemically inert materials like polystyrene. It is a solvent-free, rapid, and scalable method that allows modification at nanometer depth with minimal impact on the polymer's bulk properties. (93) Plasma treatment may be used as an effective way to both clean and activate surfaces before other treatment or deposition methods are applied. Including a cleaning method as a first step of the surface modification process, such as plasma or other techniques (solvent cleaning, abrasive blasting), is important to enhance the modification process or extend the durability of surface modifications. (92)

Chemical treatments, including wet chemical etching and functional group grafting, are employed to modify surface energy, enhance adhesion, or introduce biofunctionality. These methods often use oxidizers or acids/bases to selectively functionalize or roughen the polymer surface. (93)

Thermal treatments such as annealing can alter crystallinity and induce surface instabilities. In thin polymer films, controlled thermal annealing can generate wrinkle or ridge patterns due to differential stress relaxation, making it especially relevant for surface patterning and hierarchical structuring. (93)

Patterning methods for polymer surface modification include instability-driven approaches like dewetting and thermal or electric field gradients, as well as more controlled techniques such as photolithography, nanoimprint lithography, block copolymer self-assembly, and laser surface texturing. These techniques enable the formation of micro- to nanoscale surface structures through either spontaneous self-organization or guided processes, allowing for tailored surface topographies that enhance functionalities such as wettability, adhesion, or optical properties. (93)

These methods are often selected based on the desired surface properties and compatibility with the target application or further material processing. (93)

3.1.1 Thermal wrinkling

Thermal wrinkling can be considered a specific type of instability-induced patterning. It is a process through which wrinkles are introduced to the surface layer with the intention of altering its properties, such as enhanced adhesion, wettability, or optical performance. (94, 95) Changing the topography in this way is a technique mimicking naturally occurring phenomena - wrinkles are a repeating pattern existing in nature in various forms from nano to macro-scale, some examples include the wrinkles on the skin or the mountains. (1)

Wrinkling is a form of mechanical instability that occurs when a thin film or coating adhered to a substrate undergoes compressive stress. (94) Thermal wrinkling refers specifically to cases where this stress arises due to temperature-induced expansion or contraction, often resulting in periodic surface patterns such as waves or ridges. (95) In systems composed of a stiff thin film bonded to a softer, compliant substrate, wrinkling occurs when compressive loading leads to out-of-plane deformation, producing a pattern of undulations, which minimizes the total energy of the system. This energy minimization results from a balance between the bending energy of the stiff film and the stretching energy in the soft substrate. When the geometry or stress distribution varies along the surface, this can lead to *graded wrinkling*, where the amplitude and wavelength of the wrinkles vary spatially across the film. (94)

There are several conditions that need to be met in order to form wrinkled structures out of a top layer. First, as already indicated, a thermal/mechanical mismatch between the substrate and the top layer is necessary. (19) Second, there must be strong interface adhesion between the substrate and the regarded layer. (96) Third, the thickness of the layer that is supposed to form wrinkles must surpass a certain limit, depending on the materials and

conditions. (19, 96) Furthermore, this process depends on the specific materials used and is influenced by the temperature and duration of the thermal treatment. It is crucial for the temperature to be between the T_g and T_m of the polymer substrate so that the polymer can release tension by shrinking, which induces compression and tension between the adsorbed layer. (19, 22) Some polymers that have shrinkage ability include polystyrene, polyethylene, poly(ethylene terephthalate), and polyvinyl chloride. (22)

Wrinkles are characterized by their wavelength and amplitude. These properties are described by formulas 1 and 2. The first formula shows the relationship between the wrinkle wavelength λ , which depends on the film thickness h , where $\overline{E}_f$ and $\overline{E}_s$ represent the plane-strain modulus, each related to the Young's modulus and Poisson's ratio of the film (f) and substrate (s). (19)

$$\lambda = 2\pi h \left(\frac{\overline{E}_f}{3\overline{E}_s} \right)^{\frac{1}{3}} \quad (1)$$

The second formula shows the wrinkle amplitude A , which depends on the strain ε applied during the process and the film thickness.

$$A = h \sqrt{\frac{\varepsilon}{\varepsilon_c} - 1} \quad (2)$$

In the second equation, ε_c represents critical wrinkling strain, which depends on the modulus ratio as outlined in the following equation 3:

$$\varepsilon_c = \frac{1}{4} \left(\frac{3\overline{E}_s}{\overline{E}_f} \right)^{\frac{2}{3}} \quad (3)$$

Figure 16 illustrates a simple schematic representation of the thermal wrinkling in a thin film on a polymer substrate.

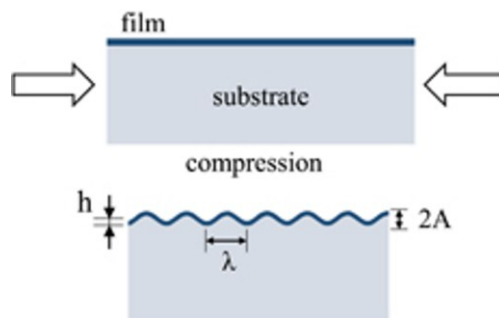


Figure 16 Formation of wrinkles via (thermal) compression (19)

As previously mentioned, this method is particularly useful for creating structured surfaces without expensive equipment, furthermore, by adjusting parameters such as introducing a gradient in elastic modulus or thickness or by micro-patterning the surface before wrinkling, the orientation and hierarchy of the wrinkles may be modified, which makes it relevant to my work on hierarchically structured polymer surfaces with nanoparticles. (19, 96)

3.2 Deposition methods

Another widely used approach to surface modification involves depositing thin layers or coatings onto a material's surface. These coatings can be metallic, ceramic, polymeric, or composite in nature. (92) Some of the most common coating methods, historically, include electro- and electroless plating, which have been used, for example, to prevent corrosion or enhance conductivity in industrial applications. (97) Conversion coatings are metal oxide layers formed through chemical or electrochemical reactions on a metal surface, serving as its protection from corrosion. (97) Physical vapor deposition (PVD) is a technique used to create thin films through physical processes such as sputtering and evaporation. (87) Similarly, chemical vapor deposition (CVD) is employed to deposit films via chemical reactions in a gaseous environment. (87) Some other commonly used methods for thin-layer depositions include spray-coating, performed by various techniques often employing thermal treatment, vacuum, plasma and other factors, dip-coating, and spin-coating. (97)

3.2.1 Spin-coating

Spin-coating is a thin-film deposition method where a small amount of a liquid material is dropped on a smooth substrate, which then rotates at high speed around an axis perpendicular to the coated plane. (97) The material spreads and drops off the substrate edges due to centrifugal forces. The device used for spin-coating is called a spincoater or a spinner. (98)

The entire process can be divided into four key stages, as illustrated in Figure 17. In the first stage (*a* in Figure 17), deposition, the coating material is dropped in the center of the substrate attached to the spincoater. The deposition can be performed either under static conditions or dynamically, when the stage with the substrate rotates at low speed. The second stage (*b* in Figure 17), spin-up, lasts from the beginning of the spinning process until a steady speed is achieved. Spin-up is followed by spin-off (*c* in Figure 17), which is characterized by a steady speed during which the film continues to thin. During spin-off, the thinning of the film is controlled by viscous forces, surface tension, and rotation rate. The final step (*d*

in Figure 17) takes place throughout the previous stages – the evaporation of the solution is considered a spin-coating stage and plays an important role in the film's thinning process. The final thickness of the spin-coated film depends on the nature of the liquid (viscosity, concentration of solid constituents, volatility of the solvent), the interfacial properties (surface tension), the environment (temperature and humidity), and the method parameters – rotation speed (measured in rotations per minute – rpm), acceleration (during spin-up, programmed as rpm/second), and rotating time. (97, 98) Finally, the thickness can be controlled by repeating the deposition process to form multi-layered systems, which can either be of the same material or composite. The lowest thickness reported to be achieved by spin-coating is 10 nm. (98, 99)

A significant disadvantage of spin-coating is the scale at which it can be used. Large substrates would be difficult to rotate at a sufficient speed to form thin layers, which limits their industrial use. Additionally, it may not be easy to control the homogeneity of multi-layer coatings. (98) Despite the obvious challenges, spin-coating has been employed industrially and in research, e.g. in the fabrication of films in electronic devices, where small size is often desirable, or in biomedical research for multi-layer biopolymer system formation. (98, 99)

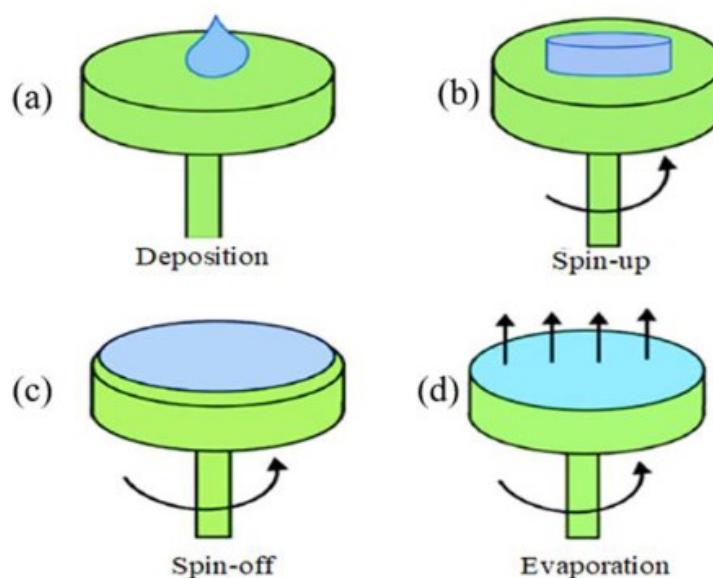


Figure 17 Four stages of spin-coating process

4 CHARACTERISATION METHODS

In this chapter, the characterization methods employed in the practical part of this thesis are described.

4.1 Surface characterization

There are several techniques suitable for polymer surface characterization, most of which are spectroscopic or microscopic, e.g. X-ray photoelectron spectroscopy for chemical composition determination (XPS), atomic force microscopy for topography investigation (AFM), ellipsometry (ELLI), surface plasmon spectroscopy (SP), infra-red spectroscopy (FTIR), X-ray and neutron reflectometry (XR, NR), scanning or transmission electron microscopy (SEM, TEM), , optical microscopy, surface tension/contact angle measurement, inverse gas chromatography (IGC), Raman spectroscopy (RS). (88) In the following sub-chapters, only SEM and FTIR are described – these were the two methods used to characterize the structured polymer surfaces prepared as described in the practical part of this thesis, due to their availability and suitability.

4.1.1 Scanning Electron Microscopy

Scanning electron microscopy (SEM) is an imaging technique based on the interaction of an accelerated electron beam with the sample to obtain information about its surface structure. The image is generated by detecting electrons emitted from the sample upon interaction with the primary beam, either secondary electrons (SE) or backscattered electrons (BSE). The electron beam raster-scans the sample surface using deflection coils and is focused by electromagnetic lenses. (100)

The electron source is typically a thermionic cathode (W, LaB₆), a cold field emission cathode (FEG) operating under ultra-high vacuum (UHV; 10⁻⁵ to 10⁻⁹ Pa), or a Schottky emission source. Emitted electrons are accelerated in an electric field with a typical accelerating voltage in the range of 0.2–30 kV. This voltage determines the wavelength of the electrons:

$$\lambda = \frac{1,227}{\sqrt{U}} [nm] \quad (3)$$

After passing through condenser and objective electromagnetic lenses, the beam is focused based on the Lorentz force principle. The magnification is controlled by the current passing through the solenoids of the lenses. Although the theoretical resolution is determined

by the electron wavelength, the practical resolution is limited by lens aberrations caused by instabilities and the geometry of the magnetic field. (100, 101)

When the primary electrons impact the sample surface, they lose energy through elastic and nonelastic interactions with sample atoms. This results in the emission of several types of signals, of which the most commonly used for imaging are:

- Secondary electrons (SE): Low-energy electrons (typically <50 eV) emitted from a very thin surface layer (1–10 nm) from the valence shells of atoms. Due to the shallow escape depth, they provide high-resolution topographic contrast. SE are typically detected by an off-axis scintillation Everhart-Thornley detector. (100, 101)
- Backscattered electrons (BSE): Primary electrons that undergo predominantly elastic (Coulombic) scattering and are redirected toward the source. Their exit energy is close to the accelerating voltage. The intensity of BSE signal depends on the atomic number, enabling compositional Z-contrast. BSE detectors are usually arranged symmetrically around the beam axis, often as segmented silicon diodes. (100, 101)

The image is formed point-by-point in synchrony with beam scanning. The signal intensity determines the brightness of each pixel. SEM resolution is limited not only by the beam diameter but also by the size of the interaction volume, which increases with accelerating voltage and decreases with atomic number. (100, 101)

Low-voltage operation (<5 kV) reduces electron penetration, which is advantageous for thin films, polymers, or non-conductive samples without coating. Higher voltages (10–30 kV) are preferred for maximum resolution in metallic samples and for chemical analysis. (100, 101)

An energy-dispersive X-ray detector (EDX or EDS) can be integrated into the SEM for elemental analysis. Characteristic X-rays are generated during atomic de-excitation following ionization of inner-shell electrons by the primary beam. The energy of the emitted photons is element-specific and allows qualitative and quantitative elemental analysis.

The spatial resolution of EDX is limited by the interaction volume (around 1 μm under standard conditions), which depends on the material density, composition, and the energy of the primary electrons. (100, 101)

4.1.2 Fourier Transform Infrared Spectroscopy

Fourier Transform Infrared (FTIR) spectroscopy is a vibrational spectroscopic technique used for molecular identification and surface analysis. It is based on the absorption of

infrared radiation by molecules, which causes transitions between vibrational energy levels. The core of FTIR instrumentation is the Michelson interferometer, where an IR beam is split into two paths and recombined after reflection from fixed and movable mirrors. The resulting interferogram contains information on all IR wavelengths simultaneously and is mathematically converted into a spectrum using the Fourier transformation. (88)

In Attenuated Total Reflection (ATR) mode, the IR beam undergoes total internal reflection within an internal reflection element (IRE), typically made of a high-refractive-index material such as silicon or germanium. An evanescent wave is formed at the interface between the IRE and the sample, penetrating a few micrometers into the sample. This allows surface-sensitive analysis of thin films, soft matter, and biological interfaces without the need for complex sample preparation. (88)

ATR-FTIR enables in situ monitoring of molecular adsorption, orientation, and conformational changes at surfaces. It is widely used in polymer science for studying adsorption kinetics, multilayer formation, and protein-surface interactions, offering high chemical specificity and quantitative capabilities. (88)

4.2 Nanomaterial characterization

Although some of the aforementioned techniques (such as electron microscopy, FTIR) are also applicable for nanomaterial characterization, some other characterization methods are suitable, especially for the chemical and structural composition of materials of nano and micro scale dimensions, such as PDA nanospheres and lysozyme nanofibers (LNF). To name a few of the most exploited ones, these methods include: energy-dispersive X-ray spectroscopy (EDX) for qualitative elemental composition analysis, X-ray diffraction (XRD) for identifying nanoparticle crystal structures, ultraviolet-visible light (UV-Vis) spectroscopy to assess optical properties and confirm NP synthesis, zeta-potential analysis, indicating colloidal stability and surface charge, or ellipsometry for thin film thickness determination. (102) Since this work is focused on biomedical applications and biopolymer particles, another method is suitable for these particles. Circular dichroism (CD) is a method used for the analysis of structural integrity and interactions with biological molecules, providing further insights into their chiral optical responses. (103)

4.2.1 Circular Dichroism

Circular dichroism spectroscopy is a non-destructive method used to characterize chiral molecules, especially biomacromolecules such as peptides, proteins and nucleic acids. The principle of the method is to measure the difference in absorbance of left- and right-handed (LCP) circularly polarized light as it passes through a sample. This difference arises because chiral molecules interact differently with each polarization state. The CD signal is typically expressed as molar ellipticity θ [deg.cm².dmol⁻¹], which is directly proportional to the difference in molar extinction coefficients (absorbance), $\Delta\epsilon = \epsilon_L - \epsilon_R$, between LCP and RCP light. The resulting spectrum, plotting ellipticity as a function of wavelength, contains valuable information about the secondary structure of proteins. Characteristic absorption bands for secondary structures of macromolecules such as α -helices, β -sheets, and random coils appear in the far-UV region (190–250 nm). The light source in a CD spectrometer is usually a deuterium lamp, with polarization modulated between LCP and RCP using a photoelastic modulator (PEM) or a birefringent crystal. A detector records the resulting intensity differences as a function of wavelength and polarization. This type of spectroscopy has a high sensitivity and temporal resolution, which allows monitoring the kinetics of structural changes of biomacromolecules. (104)

4.2.2 Dynamic Light Scattering

Dynamic Light Scattering (DLS), also referred to as photon correlation spectroscopy, is a key technique used to assess the size distribution of nanoparticles dispersed in liquids. It operates by detecting fluctuations in the intensity of light scattered by particles undergoing random thermal (Brownian) motion. These fluctuations are mathematically analyzed to extract the diffusion coefficient, which is then converted into particle size using the Stokes–Einstein relationship. DLS is especially valuable for determining the polydispersity index (PDI), a metric indicating how uniform the sample is, where lower values correspond to more uniform (monodisperse) systems. The method is widely applied to characterize nanoparticles produced via biological synthesis, such as metal nanoparticles, and is sensitive enough to monitor size changes over time or due to environmental conditions.

Complementing DLS, zeta potential analysis provides insight into the surface charge and stability of nanoparticles in suspension. The zeta potential reflects the electrical potential at the boundary of the mobile fluid layer surrounding a particle, and its magnitude correlates with the repulsive forces between particles. Measurements are typically carried out by

applying an electric field and observing the electrophoretic mobility of the particles. High absolute zeta potential values – typically above +30 mV or below –30 mV – indicate strong interparticle repulsion and hence good colloidal stability, whereas values near zero suggest a tendency to aggregate. This technique is essential for evaluating the surface chemistry and dispersion behaviour of nanoparticles, particularly those synthesized with biological agents, which can impart variable surface characteristics. (102)

4.2.3 Ultraviolet-Visible Light Spectroscopy

Ultraviolet–Visible (UV–Vis) spectroscopy is a well-established analytical technique based on the measurement of a substance's ability to absorb light in the ultraviolet and visible regions of the electromagnetic spectrum. The principle of the method relies on the excitation of electrons within a molecule: when the sample is irradiated with light of a specific wavelength, electrons may absorb energy and transition from a ground state to an excited state. The amount of light absorbed at each wavelength is recorded and used to determine the absorbance, which is mathematically related to the concentration of the absorbing species through the Beer–Lambert law. (102)

In the context of nanoparticle characterization, UV–Vis spectroscopy is frequently used to monitor the optical properties of colloidal suspensions, especially metallic nanoparticles such as silver or gold. These particles exhibit a unique phenomenon known as surface plasmon resonance (SPR), where collective oscillations of conduction electrons produce a characteristic absorbance peak in the visible region. The position and intensity of this peak are influenced by factors such as particle size, shape, and aggregation state. Additionally, UV–Vis spectroscopy can be applied to assess nanoparticle stability, detect changes over time, and estimate bandgap energy in semiconducting nanomaterials using diffuse reflectance methods. The technique's non-destructive nature, rapid measurement capability, and suitability for aqueous samples make it a valuable tool in both research and applied sciences. (102, 105)

II PRACTICAL PART

GENERAL EXPERIMENT INTRODUCTION

The objective of the practical work in this thesis was to fabricate hierarchically structured polymer surfaces for potential applications in tissue engineering. These surfaces combined micro-scale wrinkles, generated through a thermal wrinkling process, with nanoscale features introduced via bottom-up deposition of nanoparticles. These hierarchical structures were prepared on pre-stressed polystyrene (psPS) substrates, which were modified either through solvent-induced surface disruption or by spin-coating multilayer films composed of lysozyme nanofibers (LNF), hyaluronan (HA), and polydopamine (PDA) in various combinations, in both cases followed by thermal wrinkling. Polydopamine nanoparticles (PDA NPs) served as the nanomaterial to create hierarchical features on the wrinkled surfaces. All samples were characterized using scanning electron microscopy (SEM). The resulting surfaces are expected to enhance cell adhesion or facilitate the adsorption of another functional layer, leveraging the strong and universal adhesive properties of PDA, particularly when employed as a top layer.

The first experiment involved psPS wrinkling and the deposition of PDA NPs onto the psPS substrates.

In the initial stage of the experiments, it was investigated whether the surface of pre-stressed polystyrene (psPS) foil could be wrinkled by applying a solvent to induce different behaviour in its top layer, followed by thermal annealing to shrink the modified psPS substrate.

Subsequently, PDA NPs were deposited onto the psPS *in situ* by immersing the substrates in the reaction solution at the onset of the PDA NPs synthesis. The polymerization and deposition of PDA NPs thus occurred simultaneously. Two methods of synthesis were probed to evaluate the influence of reaction conditions on the amount of deposited particles and their morphology. During the reactions, the duration of immersion varied to explore the effect of PDA aging time on the particle coverage and their morphology, and to assess the ideal immersion time.

Furthermore, it was examined if a difference in topography can be observed depending on whether the deposition of PDA NPs occurs before or after the wrinkling of the psPS.

The second series of experiments focused on the thermal wrinkling of biopolymer films deposited on psPS substrates via spin-coating.

Two materials were used for the coating: LNF suspension and HA solution. LNF is supposed to have a positive charge, while HA is supposed to be negatively charged. These materials were spin-coated in alternating layers on plasma-activated psPS substrates, which provided the substrate with a negative surface charge. The alternating deposition was expected to enhance interlayer adhesion through electrostatic interactions.

Multilayer films consisting of four or five alternating layers were fabricated to investigate: (a) the effect of the outermost layer (LNF or HA) on surface properties, and (b) the influence of film thickness on wrinkle formation.

After the investigation of the wrinkling process, the next experiment was done to induce the hierarchy and to alter the surface chemical properties of the prepared substrates through *in-situ* PDA NPs deposition, which was performed in the same manner as the deposition on the bare psPS substrates. Substrates with four and five layers were immersed in the PDA NPs reaction solution for a period chosen based on the first experiments with psPS. As before, deposition was carried out both before and after the wrinkling step to evaluate the impact of sequence on surface morphology.

An additional experiment was conducted to explore an alternative method of PDA synthesis and deposition. In this case, PDA was synthesized under modified conditions using a strong oxidizing agent to accelerate the polymerization and induce the formation of a continuous film rather than discrete nanoparticles. This experiment employed an *ex-situ* approach, in which small volumes of the aged PDA reaction solution were used for spin-coating an additional layer after specific aging times.

5 MATERIALS AND METHODS

In this chapter, materials used for the modified surface preparation will be listed, and the separate methods will be described in detail.

5.1 Materials

The following materials and chemicals were used in the experiments: MilliQ water; pre-stressed polystyrene (psPS) foils; acetone ($\geq 99.5\%$, CAS No: 67-64-1); lysozyme chloride from chicken egg white (HEWL) (Grade VI, $\geq 35,000$ units/mg protein, CAS No: 9066-59-5); hydrochloric acid (37%, VWR Chemicals); hyaluronic acid (243 Da, kindly provided by Contipro a.s., Dolní Dobrouč, Czech Republic); dopamine hydrochloride ($\geq 98\%$, CAS No: 62-31-7); ammonium hydroxide (28–30%, CAS No: 1336-21-6, Thermo Fisher Scientific); ethanol, pure ($\geq 99.5\%$, CAS No: 64-17-5); sodium periodate ($\geq 99.8\%$, CAS No: 7790-28-5); and sodium thiosulfate pentahydrate ($\geq 99.5\%$, CAS No: 10102-17-7). Acetate buffer was prepared from sodium acetate ($\geq 99.0\%$, CAS No: 127-09-3) and glacial acetic acid ($\geq 99\%$, CAS No: 64-19-7). Unless stated otherwise, all chemicals were purchased from Sigma-Aldrich.

5.2 Polystyrene wrinkling after acetone treatment

Pre-stressed polystyrene sheets were cut into approximately 2×2 cm squares and cleaned by wiping with ethanol, followed by drying with compressed air. The cleaned psPS substrates were then rinsed with acetone and left to air-dry for at least 20 minutes. As acetone is a good solvent for polystyrene, it disrupted the surface, resulting in a visible change from a transparent appearance to a matte white finish. To yield wrinkled structures, thermal annealing was performed by placing the samples in an oven at 115°C for ten minutes. The formed structures were then observed using SEM.

5.3 In-situ deposition of polydopamine nanoparticles

To cover the surface of the psPS substrates, a reaction protocol for PDA NPs synthesis proposed by Ai et al. (2013) was followed, hereafter referred to as *Reaction A*. This method employed ethanol, water, and ammonia as the reaction medium. (106)

For comparative purposes, a second protocol for PDA synthesis described by Xue et al. (2016) was also tested, hereafter referred to as *Reaction B*. This method utilized a solvent mixture of ethanol, acetone, and water. (86)

Pre-stressed polystyrene samples were immersed in the solutions for PDA synthesis at the onset of the reaction (together with dissolved dopamine). The samples were immersed in the solutions for a series of times, corresponding to the aging time of polydopamine. Both reactions were run for a total of 24 hours. Therefore, various immersion times throughout the 24-hour span were chosen. The mixture of solvents with dissolved dopamine can be referred to as a solution or suspension as the reaction progresses and polydopamine forms particles that eventually aggregate.

Initially, flat (non-wrinkled) psPS substrates were used for both reaction types to compare deposition efficiency. Based on the results, *Reaction A* was selected as the more suitable method for further experiments. Therefore, all subsequent PDA NPs depositions were performed following the protocol according to Ai et al. (2013).

Second, PDA NPs were deposited onto wrinkled psPS samples. They were removed from the reaction suspension after various times.

Third, to compare the effect of the sequence of wrinkling-deposition/deposition-wrinkling, PDA NPs were deposited onto flat psPS substrates, which were subsequently wrinkled (rinsed with acetone, dried, and thermally shrunk). Multiple immersion durations were tested in parallel with previously prepared samples. This experiment was then repeated, with one additional step – the samples were rinsed with MilliQ water after they were removed from the polymerization solution to eliminate the influence of unpolymerized dopamine, solvents or any potential impurities on the wrinkling process.

After removing the samples from the solution (suspension), they were then dried in the oven at 30 °C and imaged using SEM.

5.3.1 Reaction A

Per the protocol by Ai et al. (2013), 40 mL of ethanol and 90 mL of MilliQ water (v/v = 1:1.25) were mixed, followed by the addition of 750 μ L of ammonium hydroxide to adjust the pH. After 30 minutes of stirring, the pH was measured (~ 11). Dopamine hydrochloride (0.5 g), dissolved in 10 mL of water, was then added. The concentration of DA was 3.6 mg/mL. The pH subsequently decreased to ~ 10 . The onset of polymerization was indicated by an immediate color change to pale pink, followed by a gradual transition through light brown, dark brown, and finally black. Polymerization was allowed to proceed for 24 hours.

5.3.2 Reaction B

Although water and ethanol were also used in Reaction B, acetone was additionally included, and the solvent ratios differed from those in Reaction A. Moreover, pH adjustment was achieved using TRIS buffer instead of ammonia. While the solvent ratios followed the protocol by Xue et al. (2016), the absolute volumes were modified. Ethanol (12.5 mL), acetone (12.5 mL), and MilliQ water (100 mL) were mixed in a 1:1:8 volume ratio. Subsequently, 0.125 g of dopamine hydrochloride and 750 μ L of TRIS buffer were added to adjust the pH to approximately 9. Polymerization proceeded for 24 hours.

5.4 SEM characterization of psPS modified with wrinkles and PDA

The modified psPS surfaces, prepared as described in the previous sections, were imaged using scanning electron microscopy (SEM).

Dry samples were mounted onto specimen stubs using carbon adhesive tape. Subsequently, they were sputter-coated with gold for 60 seconds at a current of 18 mA. After coating, samples were cleaned with compressed air to remove any residual particles that could damage the SEM instrument.

Imaging was performed under the following conditions: accelerating voltage of 15 kV; a combination of backscattered and secondary electron detectors (BSD + SED); high vacuum (0.10 Pa); and high frame averaging with a scan resolution of 3840×2400 pixels.

5.5 Lysozyme and hyaluronan film preparation via spin-coating

Lysozyme nanofiber suspension and hyaluronan solution were prepared and then spin-coated on psPS substrates activated via plasma treatment.

5.5.1 Lysozyme nanofibers preparation

Hen egg white lysozyme (HEWL) was dissolved in Milli-Q water to a final concentration of 2 wt% in a 15 mL centrifuge tube using a vortex mixer. The pH of the solution was adjusted to 2.0 using 1 M hydrochloric acid. The resulting solution was incubated at 90 °C for 24 hours in a thermomixer (Eppendorf) set to 400 rpm to promote fibrillation. After incubation, the suspensions were cooled to room temperature and subsequently stored at 4 °C before being used for spin-coating. (107) The suspension was characterized with DLS to assess the approximate size of the LNF aggregates and CD to characterize the LNF secondary structure.

5.5.2 HA preparation

Hyaluronic acid (HA) with a molecular weight of 243 kDa, stored in polypropylene Falcon tubes at 4 °C, was used to prepare 0.5 wt% and 2 wt% aqueous solutions in Milli-Q water. A 5 mg/mL HA solution was prepared in a glass vial by stirring with a magnetic stir bar at 300 rpm in a 39 °C water bath overnight. The same procedure was used to prepare the 20 mg/mL solution. All prepared HA solutions were stored at 4 °C until further use for spin-coating.

5.5.3 Spin-coating parameters

Spin-coating was performed using a three-step program: In the first step (ramp-up), the rotation speed increased from 0 to 2000 rpm over 20 seconds (acceleration setting 1), followed by a further increase to 3000 rpm over 10 seconds. The second step (spin-off) maintained a constant speed of 3000 rpm for 30 seconds. Finally, in the third step (deceleration), the rotation speed was reduced to 0 rpm over 13 seconds using acceleration setting 2.

Plasma-activated psPS substrates were contained in a sealed Petri dishes for no more than 7 days prior to spin-coating. The substrates were secured in the spin coater using vacuum suction. Subsequently, five drops of a 2 wt% LNF suspension were applied to the surface, and the spin-coating process was carried out as described above. Immediately following the deposition of the first layer, a solution of hyaluronic acid (HA), either 0.5 wt% or 2 wt%, was deposited using the same spin-coating parameters. (It should be noted that 0.5 and 2 wt% HA were never used in one system.) LNF and HA layers were alternately applied to form multilayered coatings consisting of either four or five total layers, denoted as 4L or 5L, respectively. In all cases, the LNF suspension was deposited as the initial layer to exploit electrostatic attraction between the positively charged LNF and the negatively charged plasma-treated psPS surface. As a result, 4L samples had HA as the topmost layer, while 5L samples had LNF. Additionally, 4L and 5L samples with LNF only were fabricated.

5.6 Lysozyme-hyaluronan film wrinkling

The psPS substrates coated with alternating LNF and HA layers (4 or 5) were thermally shrunk to induce the wrinkling of the spin-coated films. The samples were placed on a glass Petri dish and put into an oven set at 120 °C for 15 minutes. The wrinkled samples were

characterized by FTIR to assess the chemical composition of the top layers and compare LNF vs HA and 0.5 wt% HA vs 2 wt% HA. The samples were then imaged with SEM.

5.7 In-situ deposition of polydopamine onto lysozyme-hyaluronan films

The samples modified with spin-coated LNF and HA were further modified with polydopamine. The PDA deposition was carried out using *Reaction A*, as previously described in Section 5.3 and based on the protocol by Ai et al. (2013). Briefly, the reaction mixture consisted of ethanol, water, and ammonia, into which dopamine was added at a final concentration of 3.6 mg/mL. In this experiment, both four-layered and five-layered films composed of 2 wt% LNF and 2 wt% HA were used. Following deposition, all samples were rinsed thoroughly with Milli-Q water to remove unreacted components and residual reaction medium.

The samples with spin-coated LNF-HA films were either first immersed in the PDA synthesis solution to wrinkle them after the deposition or wrinkled prior to the PDA deposition.

After the samples had air-dried, the coverage of the LNF-HA surfaces with PDA was examined using SEM.

5.8 Ex-situ deposition of polydopamine onto lysozyme-hyaluronan films

The following procedure was inspired by the study of Le et al. (2019), which explored the deposition of polydopamine onto various substrates employing the spin-coating technique. Based on their findings, a strong oxidizing agent was used to speed up the process of PDA deposition. (84) Other advantages of this method include less material loss, fast film formation and close-to-neutral pH of the reaction conditions.

Four-layered and five-layered films of 2 % LNF and 2 % HA were modified in this experiment by spin-coating of differently aged PDA solutions, as shown in Figure 18, followed by thermal wrinkling. The morphology of these surfaces was examined by SEM. The size of differently aged PDA particles was measured using DLS.

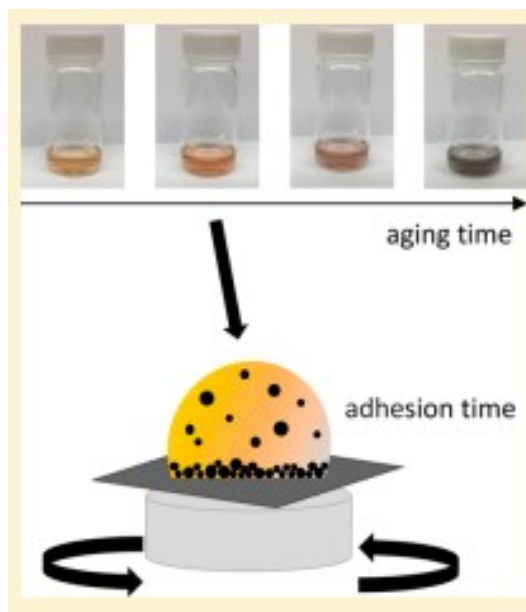


Figure 18 A picture summarizing the steps of ex-situ PDA deposition and spin-coating (84)

5.8.1 Buffer preparation

The reaction pH was maintained at 6.0 using a 0.25 M acetate buffer. The buffer was prepared by mixing aqueous solutions of acetic acid and sodium acetate trihydrate in appropriate proportions.

5.8.2 Polydopamine synthesis

PDA was synthesized ex situ in a glass vial under continuous stirring. The reaction pH was maintained at 6.0 using the previously prepared acetate buffer. Dopamine hydrochloride was used at a concentration of 4 mg/mL, and sodium periodate (NaIO_4) was used as the oxidizing agent at a 2:1 molar ratio relative to dopamine, corresponding to a concentration of 8 mg/mL.

The reaction mixture was prepared by dissolving 80 mg of dopamine hydrochloride and 160 mg of sodium periodate in 20 mL of acetate buffer.

5.8.3 Inactivating agent preparation

A quenching solution was prepared using sodium thiosulfate pentahydrate in a 1.2-fold molar excess relative to sodium periodate. The final concentration was 11 mg/mL, and 15 mL of the solution was prepared for subsequent use.

5.8.4 Spin coating of polydopamine solution

A small amount of the solution was removed after various aging times (1 min, 5 min, 30 min, 60 min, 22 h), and 188 μL (corresponding to five drops) was deposited onto the LNF-HA films.

The suspension was left to adhere to the surface for 2 minutes (defined as the adhesion time), after which the spin-coating process was immediately performed using the parameters described in chapter 5.7.

5.8.5 DLS characterization of the aged PDA suspension

After various aging times (1 min, 5 min, 30 min, 60 min, 20 h), a small amount of the aged suspension was removed, and the oxidizer was inactivated with sodium thiosulfate and diluted in the acetate buffer to a final concentration of 0,025 wt%. Then, the size of the PDA particles was measured with a zetasizer (Malvern Panalytical.)

5.8.6 Wrinkling of spin-coated films

Following PDA deposition, the spin-coated films were thermally wrinkled by heating in an oven at 120 °C for 15 minutes. After cooling to room temperature, the samples were rinsed with sodium thiosulfate solution to prevent further oxidation.

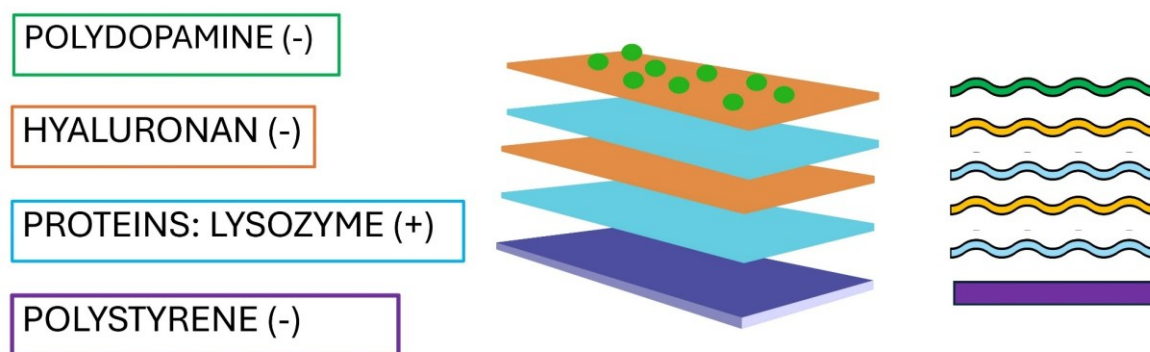


Figure 19 General simplified scheme of psPS modifications using spin-coating, PDA deposition and thermal wrinkling

5.9 Characterization methods of spin-coating-modified samples

All samples prepared as previously described were imaged using SEM as the main characterization method. Additional characterization methods such as FTIR, CD and DLS were used for some samples.

5.9.1 SEM characterization

The samples were attached to the SEM stubs by carbon tape. The samples were imaged without prior sputter-coating. The SEM was operated under low vacuum, cooled by water. The spot intensity was 4-5.5, and the voltage of 10-12.50 kV was used. The SE detector was used for image acquisition.

5.9.2 FTIR-ATR measurements

The prepared samples of LNF and LNF-HA wrinkled on psPS were analyzed using infrared spectroscopy. Measurements were carried out using a Nicolet iS5 spectrometer in attenuated total reflectance (ATR) mode with a germanium crystal. Prior to measurement, each sample was cleaned and placed on the crystal with the top side facing down. Key measurement parameters that influence the quality of the resulting spectrum, particularly the signal-to-noise ratio, were adjusted beforehand. The spectra were recorded using 64 scans at a resolution of 4 cm^{-1} (the spacing between spectral bands) over a wavenumber range of $4000\text{--}400\text{ cm}^{-1}$.

5.9.3 CD

To assess the secondary structure of the prepared LNF, circular dichroism (CD) measurements were performed in the wavelength range of 190–250 nm. The LNF suspension was diluted from an initial concentration of 20 mg/mL to 0.1 mg/mL. Three measurements were carried out to obtain an average spectrum.

5.9.4 DLS

The size and zeta potential of the prepared nanoparticle suspensions (LNF) were characterized using dynamic light scattering (DLS) on a Zetasizer Nano ZS90 instrument (Malvern, UK), which provides intensity-weighted size distributions of all particle populations present in the analyzed sample. The resulting particle size was expressed as the hydrodynamic diameter (RH), also known as the z-average (nm). Measurements were

conducted at a scattering angle of 173° . The instrument is equipped with a 4 mW helium–neon laser operating at a wavelength of 633 nm.

For size measurements, the prepared LNF suspension was diluted to a concentration of 0.25 wt% using HCl solution adjusted to pH 2. Each sample was measured in triplicate. From these replicates, the hydrodynamic diameter and polydispersity index (PDI) were determined.

For zeta potential measurements, a suspension with a concentration of 0.25 wt% was prepared, to which NaCl was added to reach a final ionic strength of 10 mM. LNF suspension was diluted using HCl solution adjusted to pH 2.

6 RESULTS AND DISCUSSION

In this chapter, the results of individual experiments are presented and discussed. Most of the characterizations were based on SEM imaging; however, additional material analyses were performed when suitable.

6.1 In-situ polydopamine deposition on wrinkled polystyrene

The psPS foils rinsed with acetone were thermally wrinkled in an oven at 115 °C for 10 minutes. The process was verified using SEM imaging. In Figure 20, the picture on the left (a) depicts a flat surface and the edges of a psPS foil cleaned with ethanol rinsing, wiping, and compressed air drying. The figure on the right (b) illustrates the successful wrinkling of the psPS surface.

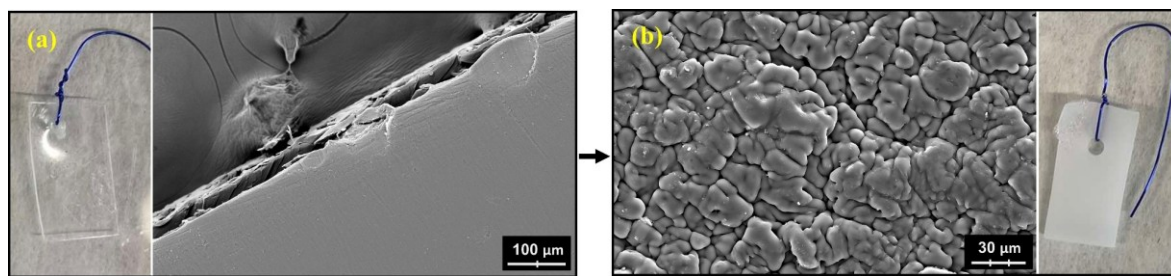


Figure 20 Camera photographs and SEM images of psPS before (left) and after (right) acetone-thermal wrinkling

The width of the observed wrinkles is in units of tens of micrometres. Photographs of the samples before and after the acetone-temperature treatment are also included to demonstrate the notable changes in the optical properties of the samples. During the thermal treatment, the psPS samples shrink by about 40 % and their thickness increases.

(Furthermore, it was shown that the uniformity of the wrinkle size cannot be precisely controlled using the acetone-rinsing method, probably due to the different evaporation time across the sample surface, especially on the edges.)

6.1.1 Comparison of Reaction A and Reaction B

Flat, clean psPS samples were submerged in two different solutions for polydopamine synthesis, referred to as *Reaction A* and *Reaction B*, to deposit PDA nanoparticles and to compare the efficiency of the two methods. Samples were removed at different time intervals, after 2, 4, 6, 8, 22, and 24 hours, and the extent of surface coverage was assessed.

It was observed that the samples were covered with a gradually darker layer with

increasing immersion time, as illustrated by Figure 21, corresponding to the SEM images.

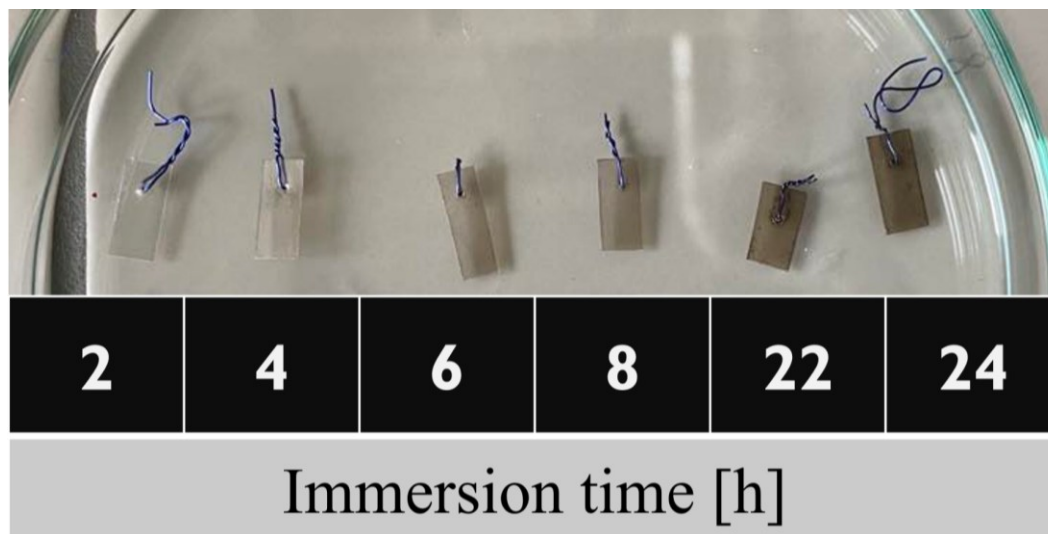


Figure 21 Photograph of psPS samples after different immersion times in *Reaction A*

In both *Reaction A* and *Reaction B*, longer immersion times led to increased PDA deposition. However, samples immersed in *Reaction A* exhibited better and more uniform coverage.

Figure 22 presents SEM images of substrates immersed in *Reaction A* for various durations. Figure 23 compares the samples immersed in *Reaction A* and *Reaction B* for 22 hours. Based on previous studies by Ai et al. (2013) and Xue et al. (2016), a significant amount of PDA deposition was expected after 22 hours, as the typical reaction time is approximately 24 hours. This expectation was confirmed for *Reaction A*; however, the samples treated in *Reaction B* were covered with noticeably less PDA. Moreover, the deposited PDA in *Reaction B* formed visible aggregates rather than a uniform layer. Therefore, further experiments were conducted using the synthesis solution from *Reaction A*. The immersion time of 22 hours was considered to be enough for relatively homogeneous coverage before big aggregates started to form and merge into film.

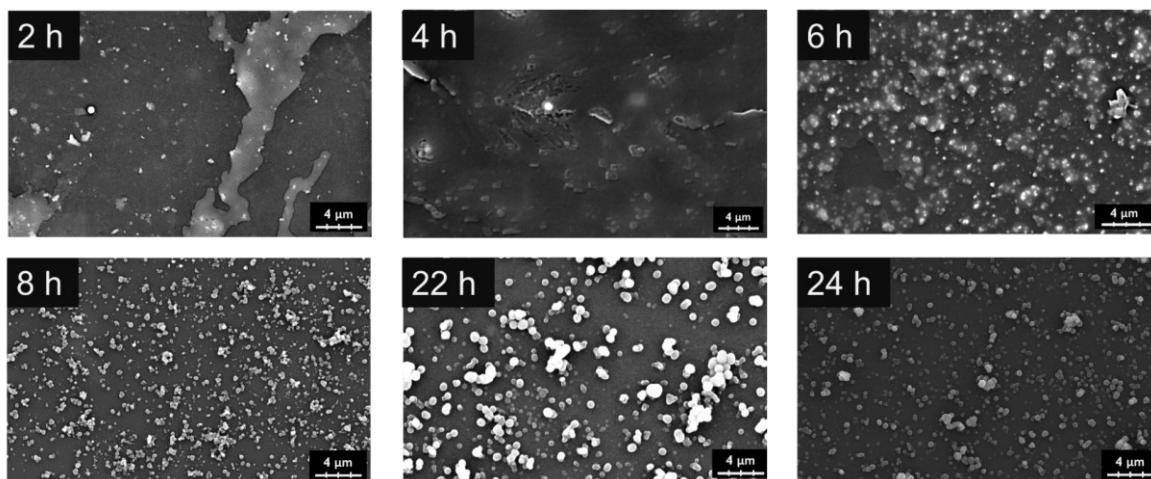


Figure 22 SEM images of flat psPS modified with PDA via *Reaction A* for various immersion times (2 to 24 hours)

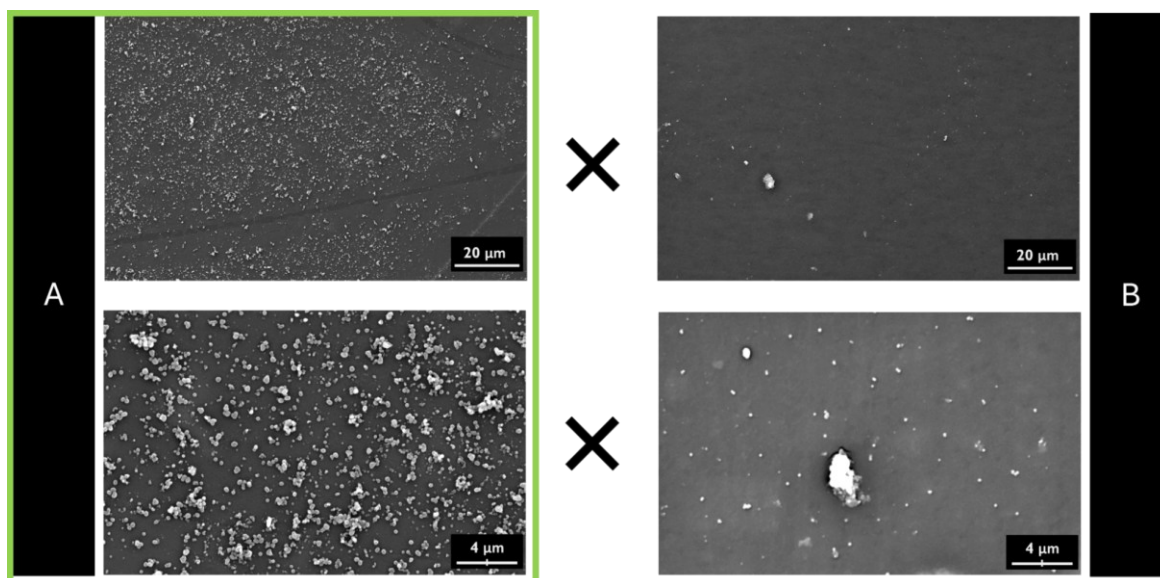


Figure 23 SEM images of flat psPS substrates with PDA NPs deposited after 22 h immersion in *Reaction A* (left) or *Reaction B* (right)

6.1.2 Polydopamine deposition after wrinkling

Thermally wrinkled psPS substrates were submerged in a Reaction A solution for PDA deposition. Figure 24 illustrates the general before and after deposition topography imaged by SEM.

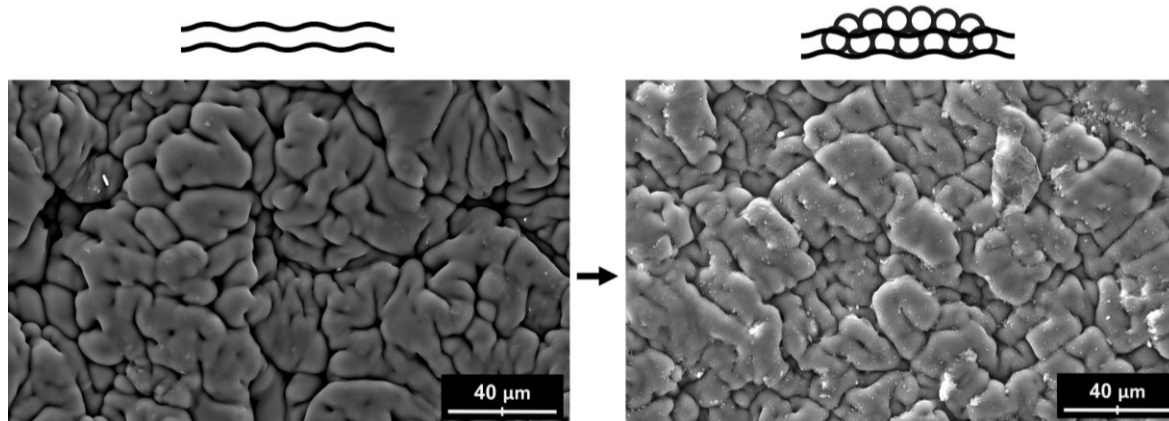


Figure 24 SEM image of before and after PDA deposition on wrinkled psPS

Successful PDA NPs deposition was observed on the wrinkled psPS surfaces.

Samples were removed at three different time intervals, which were 4, 6, and 24 hours. Then, the extent of surface coverage and potential other morphological changes were assessed by SEM, as shown in Figure 25.

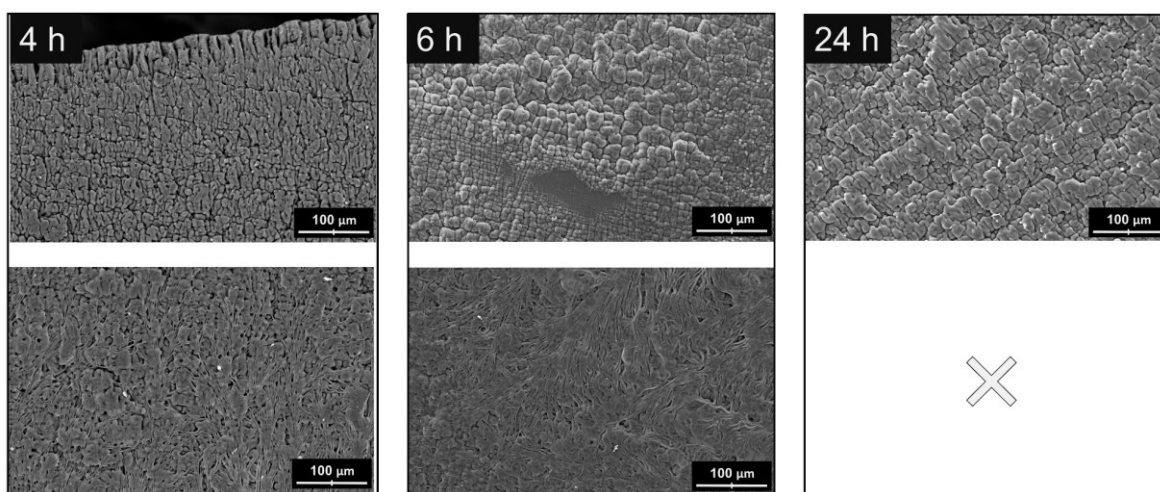


Figure 25 SEM images of wrinkled psPS followed by PDA deposition for 4, 6, and 24 h

No changes in wrinkle morphology by the reaction conditions of PDA synthesis were observed. The PDA coverage increased with time, as shown in greater detail in the following Figures (26-28).

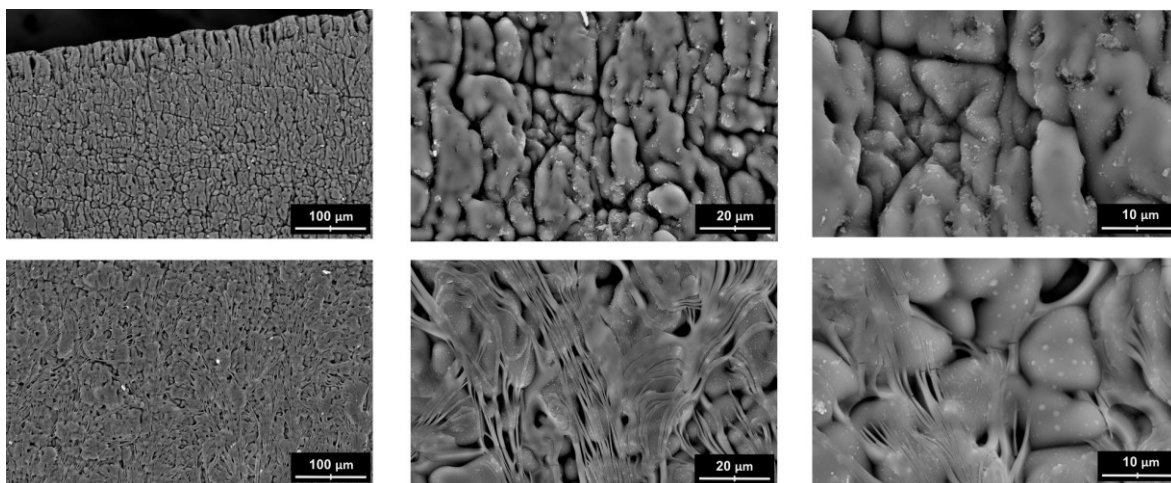


Figure 26 SEM images of wrinkled psPS modified with PDA (4 h), bottom row depicts areas covered with “fibrillar film” structures; (the scale-bar is 100, 20 and 10 μm from left to right)

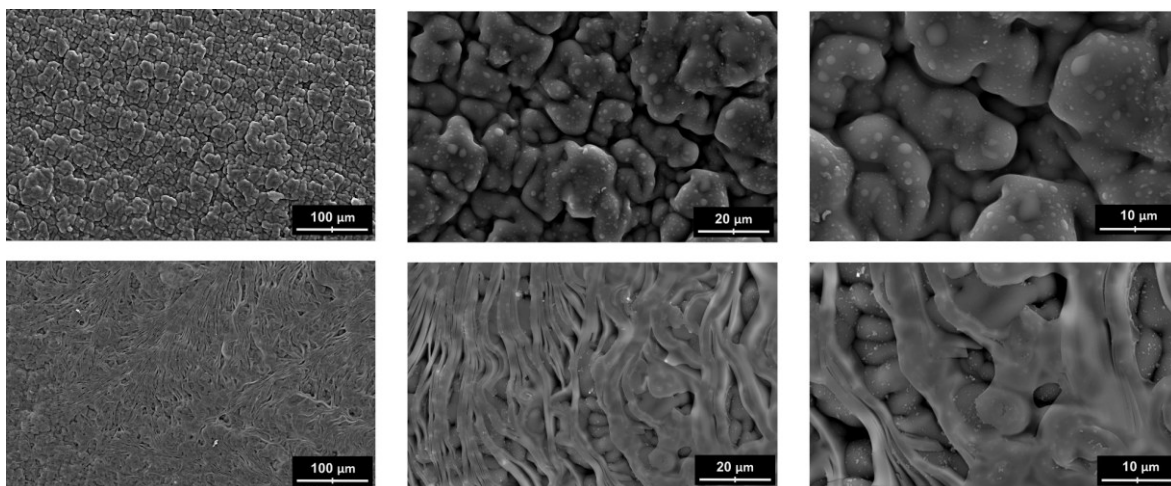


Figure 27 SEM images of wrinkled psPS modified with PDA (6 h), bottom row depicts areas covered with “fibrillar film” structures; the scale-bar is 100, 20 and 10 μm from left to right

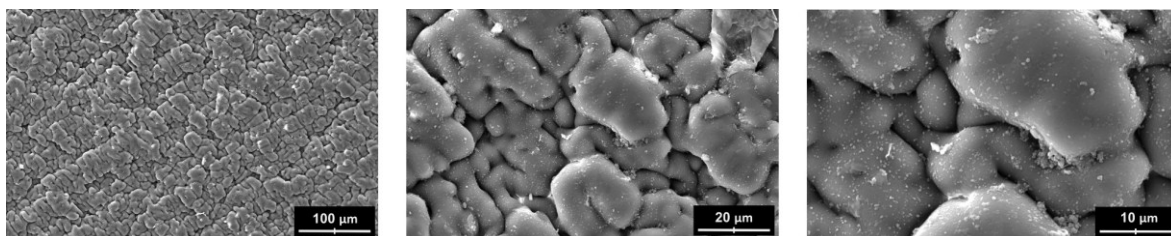


Figure 28 SEM images of wrinkled psPS modified with PDA (24 h); the scale-bar is 100, 20 and 10 μm from left to right

An unexpected morphology was observed on samples removed after 4 (Figure 26) and 6 (Figure 27) hours, which could be described as a “fibrillar film.” These “fibrillar films” formed branch- or flower-like structures on top of the wrinkled structures. It was suggested

that these were actually an unpolymerized dopamine. This idea was supported by the fact that after longer immersion times (24 h), when the dopamine should be mostly polymerized according to the protocols for PDA synthesis that were followed (Ai et al., 2013), these structures were no longer found on the imaged samples.

6.1.3 Polydopamine deposition followed by wrinkling

As described earlier, cleaned flat psPS substrates were submerged in the PDA synthesis solution and deposited with PDA NPs. Subsequently, the samples were rinsed with acetone, allowed to air-dry, and thermally wrinkled. The acetone did not remove the brown to black coating on the samples, as further confirmed by SEM imaging. Figure 29 depicts the general before and after the wrinkling of this experiment.

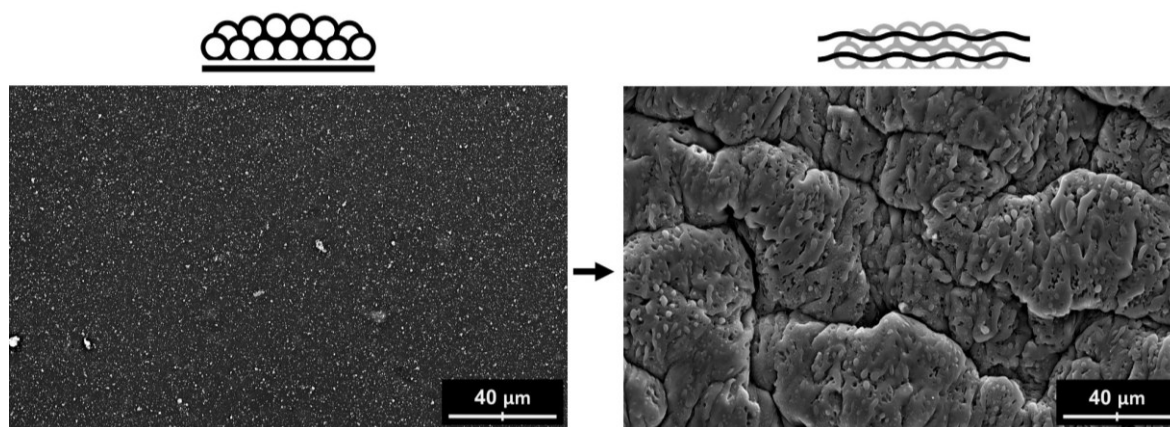


Figure 29 SEM images of before and after wrinkling of psPS with deposited PDA NPs (left image un-wrinkled with PDA, right image after wrinkling)

The samples were removed from the reaction solution for PDA NPs synthesis after 2 to 24 hours. Following the thermal wrinkling, the differences in topography between the samples immersed for the set time intervals were examined using SEM, as shown in Figures 30 and 31 below.

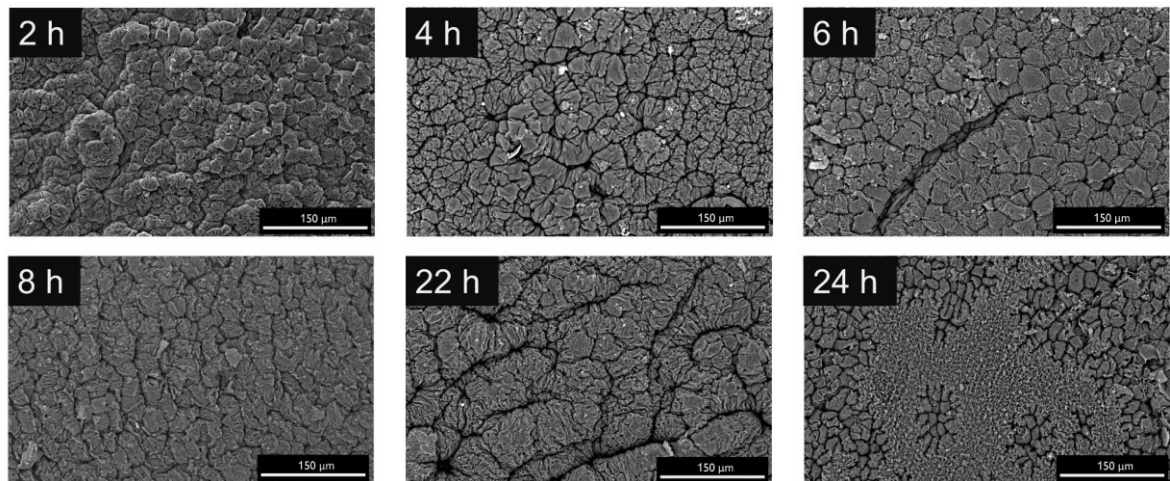


Figure 30 SEM images of psPS modified with PDA NPs' deposition (immersion times 2, 4, 6, 8, 22, and 24 hours), followed by wrinkling; the scale-bar is 150 μm

After higher deposition times (24 h), a film was observed on the wrinkled surfaces, as shown in Figure 30. The film structure was imaged with a higher magnification as depicted in Figure 31 below.

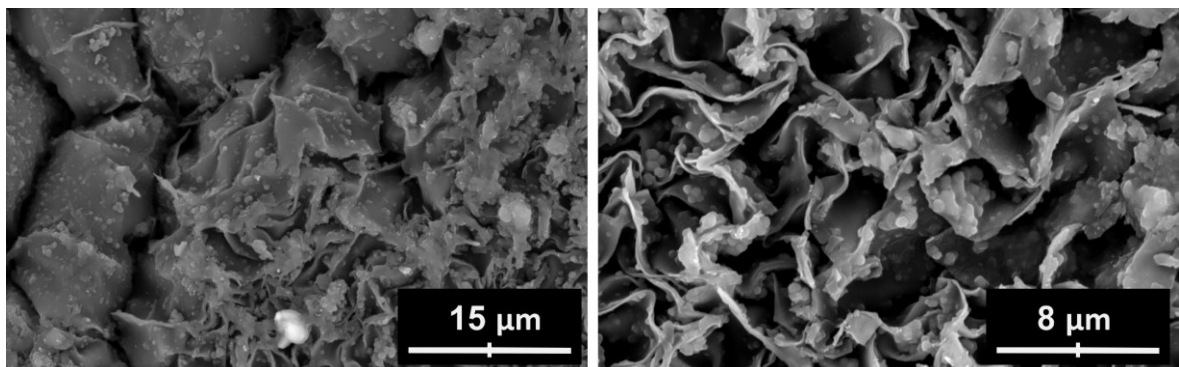


Figure 31 SEM images of PDA NPs and film on psPS modified with PDA and wrinkling; (the scalebar is 15 μm on the left image, 8 15 μm on the right image)

It was hypothesized that longer deposition times allow the polydopamine nanoparticles to merge and form a continuous layer, as supported by several publications, for example, by Kwon and Bettinger (2018). (108)

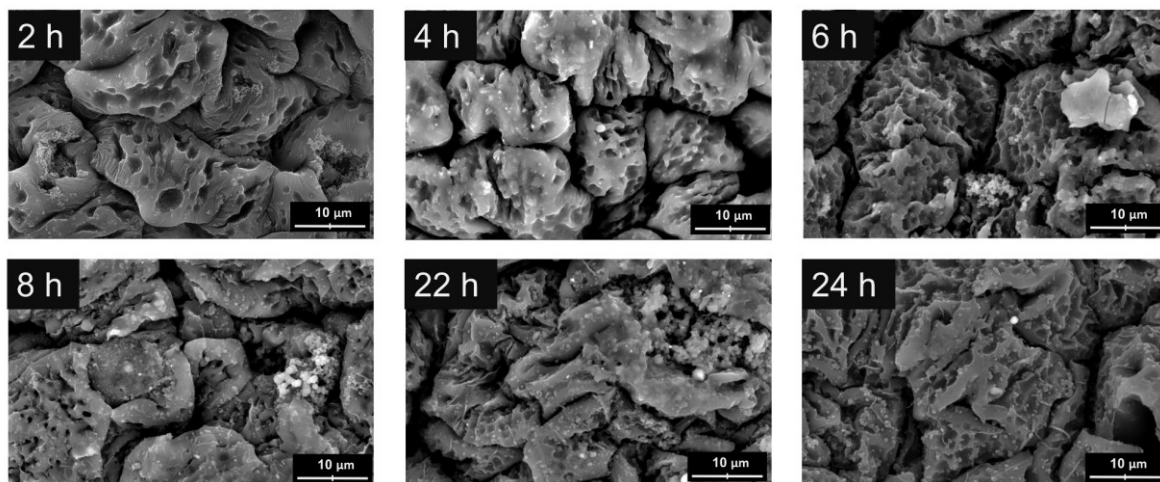


Figure 32 SEM images of psPS modified with PDA NPs' deposition (immersion times 2-24 hours), followed by wrinkling; the scale-bar is 10 μm

At higher magnification, pores ranging in size from hundreds of nanometers to a few micrometres (estimated based on SEM scale bars) were observed on the surface (Figure 32). Although it is not completely clear what caused their formation, it was hypothesized that some of the PDA NPS of spherical shape fall off the surface during the thermal shrinking process, resulting in holes in the surface. However, if the samples were rinsed with water after removal from the PDA synthesis solution before wrinkling, the amount of observed pores notably decreased, as demonstrated in Figure 33.

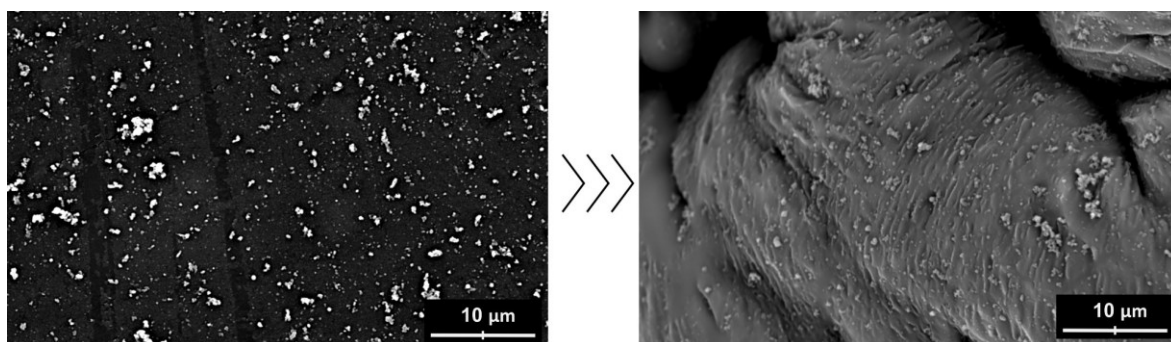


Figure 33 SEM images of psPS modified with PDA, rinsed with water and wrinkled (left image before wrinkling, right image after wrinkling)

6.2 Spin-coating of lysozyme and hyaluronan followed by wrinkling

This section discusses the results of material, surface and nanoparticle characterization.

6.2.1 Chemical composition of spin-coated and wrinkled samples

The samples with variants of layer spin-coated to form film were wrinkled and analyzed with FTIR to assess differences between films with either LNF or HA as the top layer, with HA concentrations of 0.5 wt% or 2 wt%, and films composed solely of LNF. The LNF concentration was consistently 2 wt% across all samples.

Spectra in Figure 34 are labelled according to the following naming convention: **concentration1_layer1_concentration2_layer2_number of alternating layers (L)_thermal wrinkling (TW)**, describing the sequential composition of the psPS surface modification.

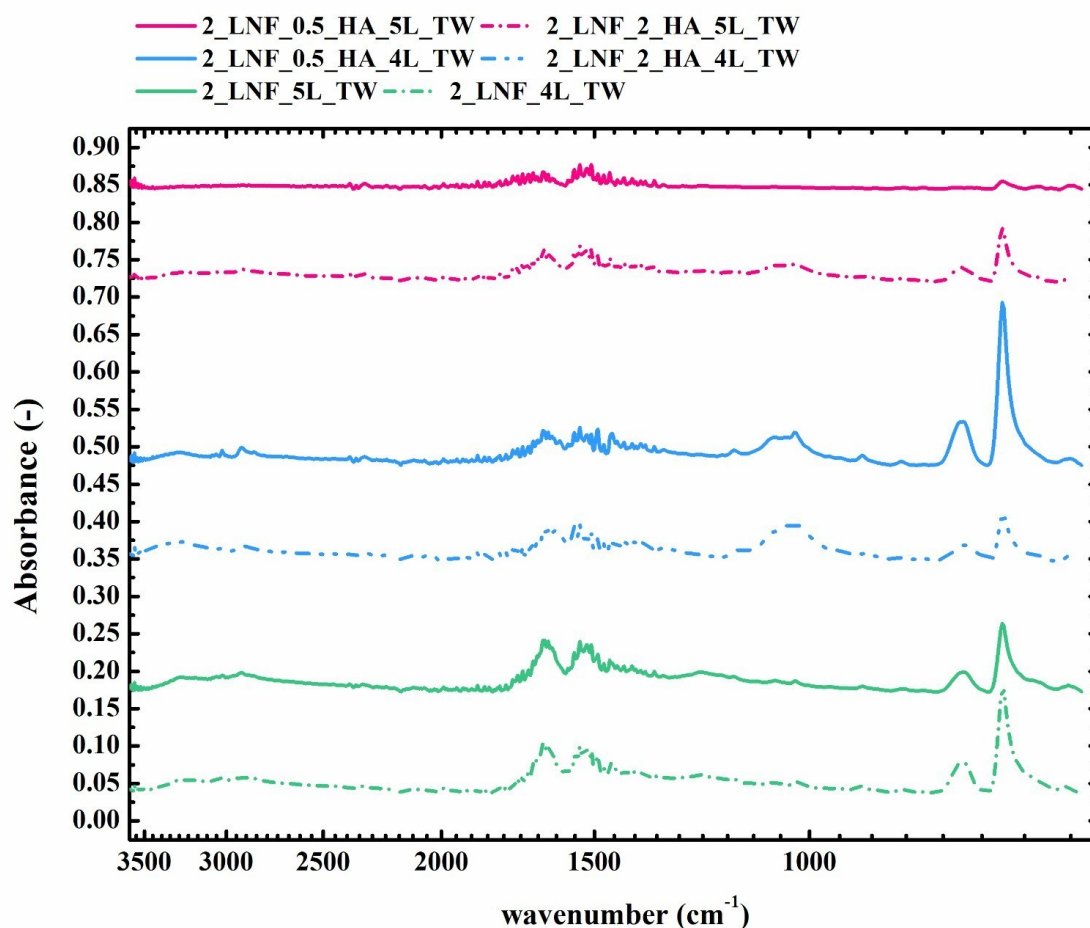


Figure 34 FTIR spectra of spin-coated and wrinkled samples (psPS-LNF-HA)

The **green spectra** correspond to LNF-only samples with 4 and 5 layers, showing consistent peak positions and comparable intensities, indicating reproducible film formation.

The **blue spectra** represent samples with HA as the top layer. The solid blue line indicates the sample with 0.5 wt% HA, while the dashed blue line represents the sample with 2 wt% HA. The higher intensity of certain peaks (between 600-400 cm^{-1}) in the solid blue spectrum suggests that the IR beam penetrated more effectively through the less concentrated HA layer, revealing signals from the underlying LNF.

The absorption band 950 – 1200 cm^{-1} can be assigned to polysaccharides, i.e. HA, which is consistent with the fact that it does not occur in spectra with pure LNF. (109) A similar peak at approximately the same wavenumber is also observed in the **dashed pink spectrum**, which corresponds to the sample with LNF as the top layer and 2 wt% HA underneath. Two possible explanations can be proposed: first, the top LNF layer may be sufficiently thin to allow infrared light to penetrate through to the underlying concentrated HA; second, due to the inherent challenges in achieving uniform coverage during the spin-coating process, the measurement may have been taken from a region with incomplete LNF coverage. The **solid pink spectrum** corresponds to a sample with LNF as the top layer and 0.5 wt% HA beneath it, and does not exhibit the same peak, supporting the hypothesis that the observed signal originates from the concentrated HA layer.

6.2.2 Morphology of spin-coated and wrinkled samples

SEM figures of samples prepared via spin-coating of LNF and HA on psPS substrates were acquired.

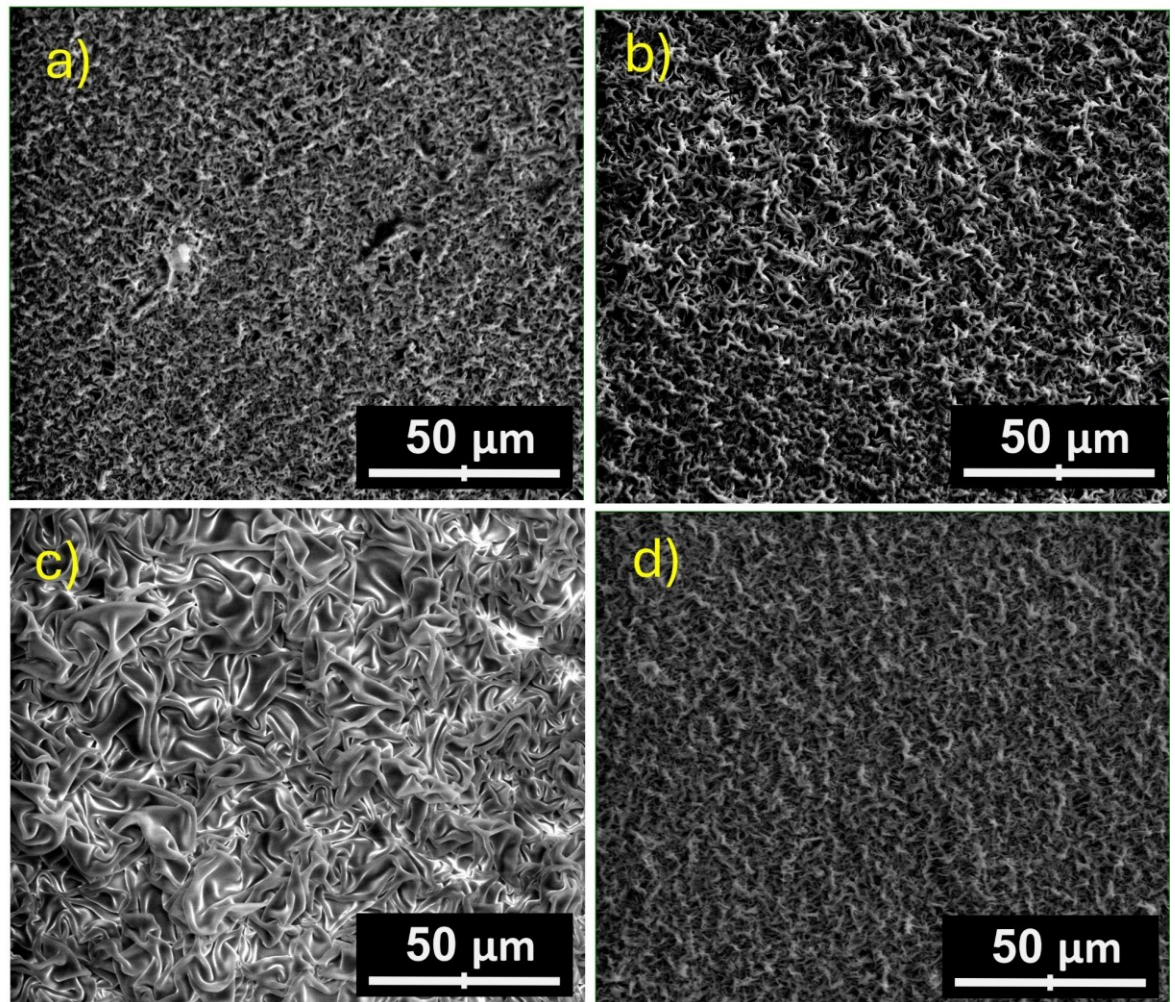


Figure 35 SEM image of thermally wrinkled samples of spin-coated layers:

a) 4L, 2 % LNF + 0.5 % HA; b) 5L, 2 % LNF + 0.5 % HA;

c) 4L, 2 % LNF + 2 % HA; d) 5L, 2 % LNF + 2 % HA

It can be deduced that the wrinkle size depends on the thickness of the film, which is bigger in the case of more concentrated HA, which corresponds with the wrinkle-formations theory mentioned in the theoretical part of this thesis. No significant differences were observed between the 4L and 5L samples. To ensure smoother and wider wrinkles, only 2 wt% HA was used in the sub-sequent experiments.

The following Figure 36 depicts wrinkles on psPS coated with four layers of alternating LNF and HA.

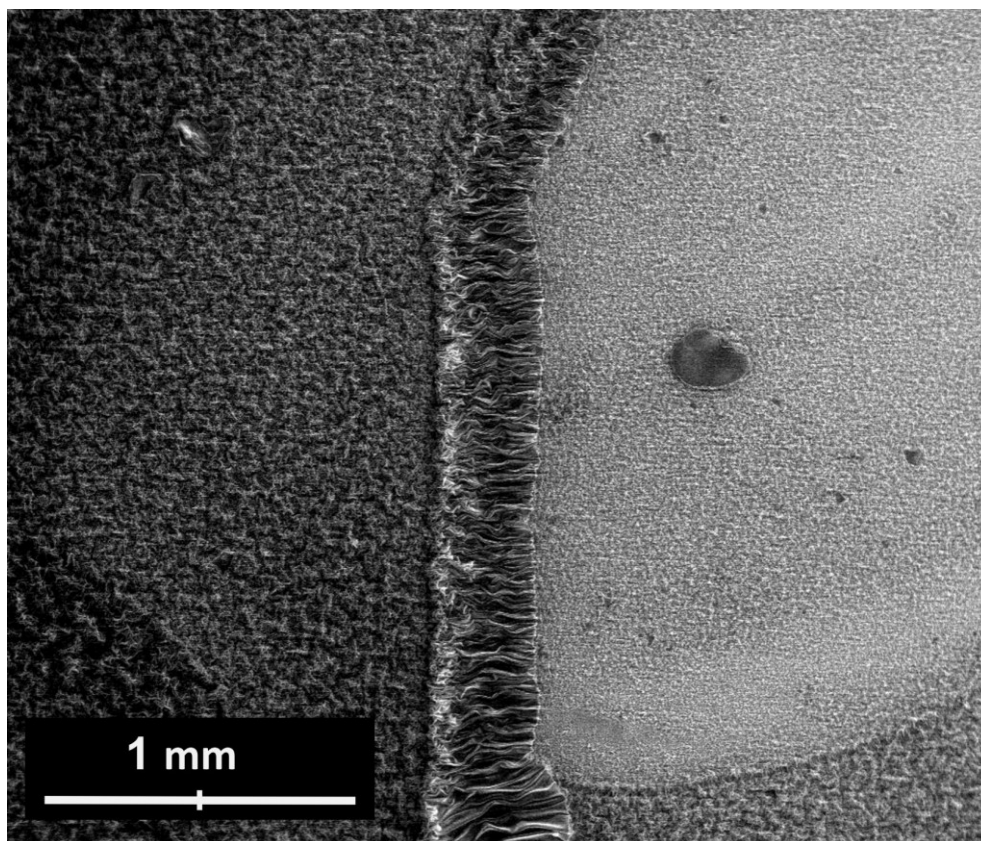


Figure 36 SEM image of inhomogeneity on psPS coated with LNF and HA, 4 layers, wrinkled

It was observed that significant differences were present in the samples, which were attributed to the process of dropping the solutions onto the substrates in a non-confluent manner before spin-coating, resulting in areas of varying thickness. The borders between the areas of different thickness represent the boundaries between separate drops of coating solutions. For future experiments, this will be corrected by allowing all drops to land in the central area of the samples. For the experiments presented, it must be noted that wrinkle wavelength may differ across the samples regardless of the number of spin-coated layers.

To investigate whether LNF is able to form wrinkled film on its own, 4 layers of 2% LNF were spin-coated and thermally shrunk; the SEM image is in the following figure 37.

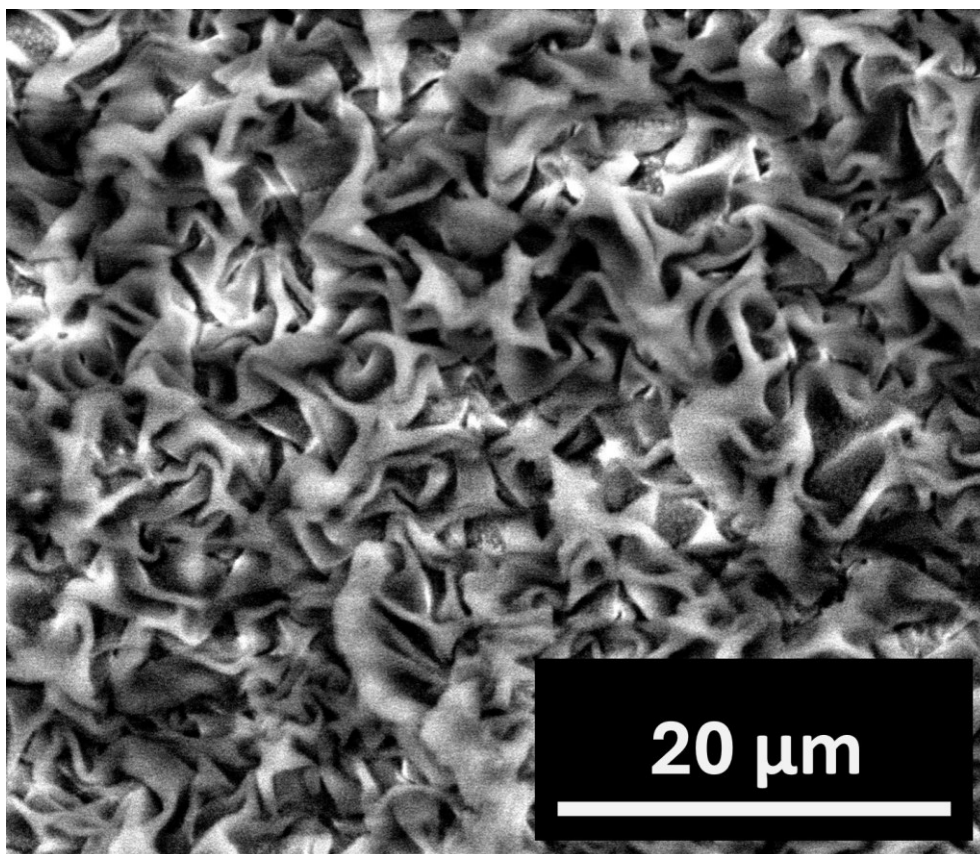


Figure 37 SEM image of LNF, 4 layers, wrinkled

Wrinkles of similar shape to those in the previous experiments were observed while using only LNF to coat the psPS substrates.

6.2.3 Size and zeta-potential of LNF

As previously mentioned, the average particle size and zeta potential of LNF in suspension were measured with DLS. It was found that z-average of LNF suspensions corresponds to a size of 244 ± 16 nm. Moreover, a narrow, unimodal particle size distribution was confirmed in the sample.

The zeta potential, which reflects the electrostatic repulsion or attraction between particles in suspension, was measured for the LNF suspension and found to be 40 ± 2 mV at pH = 2. This value indicates relatively high colloidal stability, as the particles are sufficiently charged to repel each other and resist aggregation. Furthermore, since the measurement was conducted under acidic conditions (pH = 2), the particles are expected to remain well-dispersed due to adequate surface charge.

6.2.4 Amyloid structure of LNF

As previously mentioned, the secondary structure of the prepared lysozyme nanofibrils (LNFs) was confirmed using circular dichroism (CD) spectroscopy. Amyloid fibrils, which result from the self-assembly of lysozyme molecules fragments, are typically characterized by a high content of β -sheet structures, as discussed in the theoretical section of this thesis.

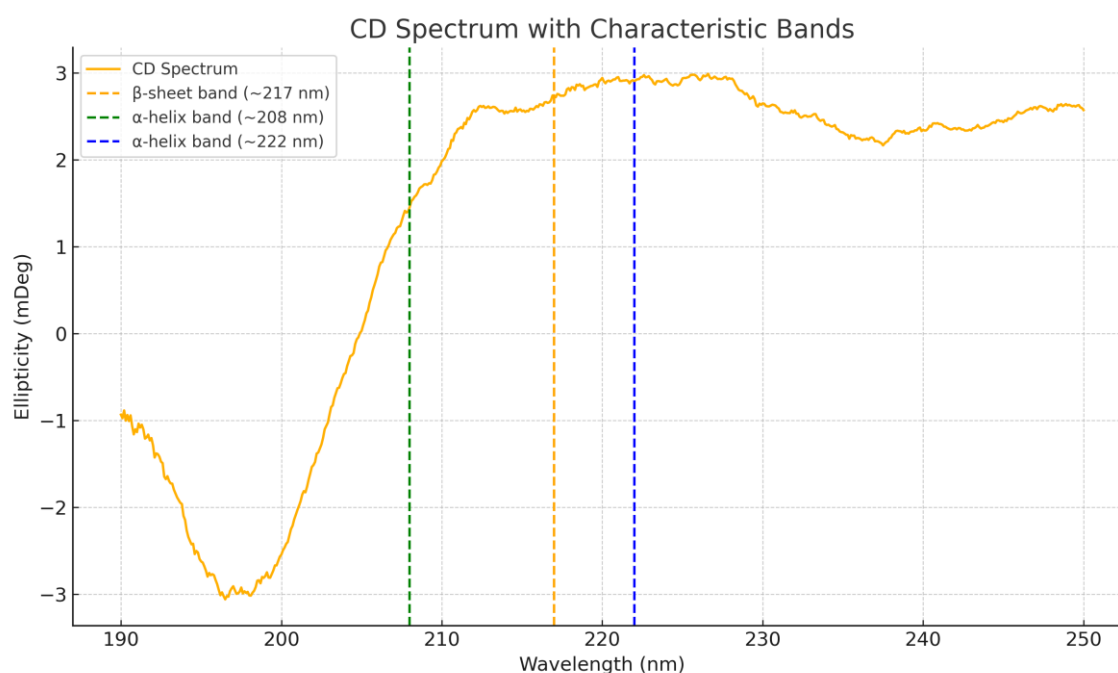


Figure 38 Ellipticity of prepared LNF measured by CD, with characteristic amyloid bands

Figure 38 shows the CD spectrum of the prepared LNFs, displaying ellipticity as a function of wavelength, with characteristic spectral regions corresponding to protein secondary structures highlighted. The α -helix structure is typically associated with two bands in the regions of 204–212 nm and 219–225 nm, while the β -sheet structure is expected within the range of 213–218 nm. The integration of ellipticity across these intervals yielded absolute values of $-28.2 \text{ mDeg}\cdot\text{nm}$ for the α -helix contribution and $-13.1 \text{ mDeg}\cdot\text{nm}$ for the β -sheet contribution. Based on these results, the estimated secondary structure composition of the sample consists of approximately 68.2% α -helix and 31.8% β -sheet structures. These findings confirm the presence of β -sheet-rich structures, supporting the successful formation of amyloid fibrils in the prepared samples. (104)

6.3 In-situ polydopamine deposition on LNF-HA surfaces

Samples coated with LNF and HA films were immersed in the Reaction A solution for PDA nanoparticle synthesis, both with and without prior thermal wrinkling. The LNF-HA films on samples that were not thermally treated before immersion dissolved immediately upon contact with the solution mixture, resulting in PDA nanoparticles adhering directly to the psPS surface. Therefore, this method was deemed unsuitable. On the other hand, samples that were thermally wrinkled prior to the PDA depositions were proven to be relatively “immune” to the reaction conditions for PDA synthesis. To simplify the experiment by excluding one variable, only one immersion time was chosen based on previous experiments and all the samples were removed after 22 hours of reaction.

The morphological changes of the wrinkled films were examined using SEM imaging, as shown in the Figures below. The two following Figures (39 and 40) show the results of the same experiment, which was repeated.

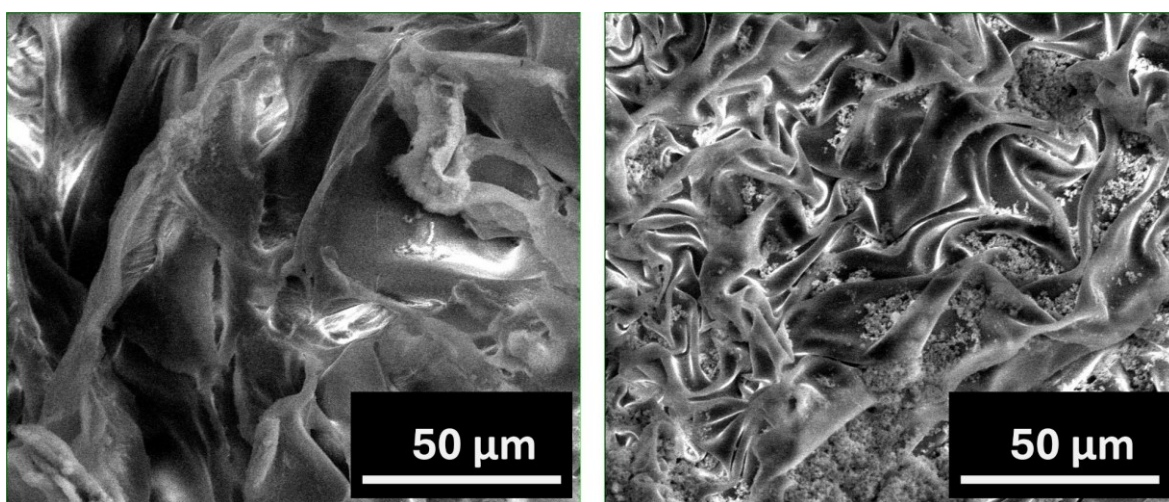


Figure 39 SEM images of LNF-HA four layer (left) and five layer (right) wrinkled films with deposited PDA NPs

The PDA NPs deposition was assessed to be very low for the samples with HA as a top layer, while the samples with LNF as a top layer were covered with PDA NPs that were forming aggregates or “islands” of almost continuous layers. The sample with HA as a top layer seems to be slightly disrupted by the reaction conditions; however, the wrinkles retained their shape in most areas.

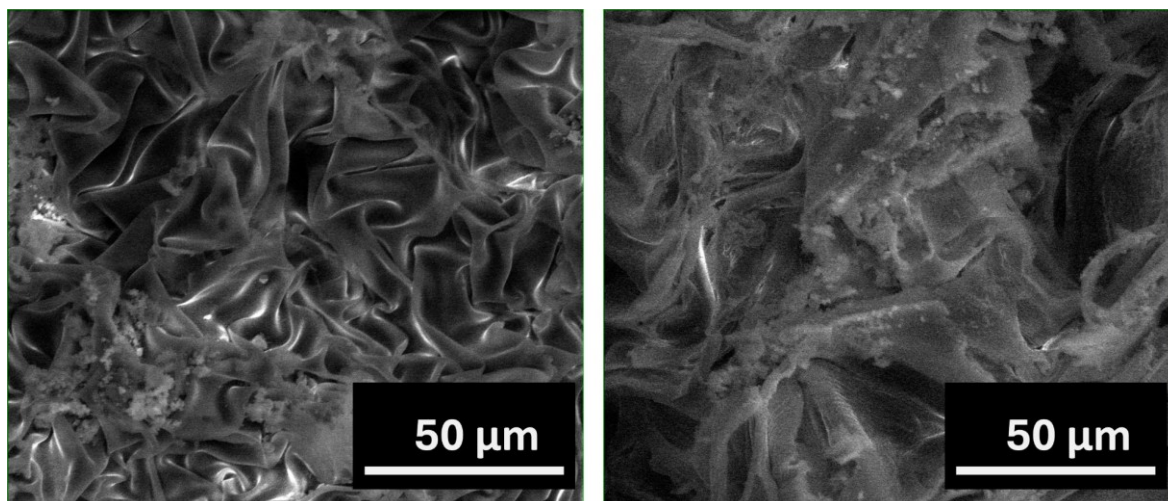


Figure 40 SEM images of SEM images of LNF-HA four-layer (left) and five-layer (right) wrinkled films with deposited PDA NPs (repeated experiment)

When the experiment was repeated, both samples were coated with PDA NPs; however, the coating was non-homogeneous. The PDA appears to be forming partially continuous layers, while other areas were almost bare. This may be due to different interactions between the spin-coated films compared to the bare psPS or due to variations in wrinkle shapes and sizes.

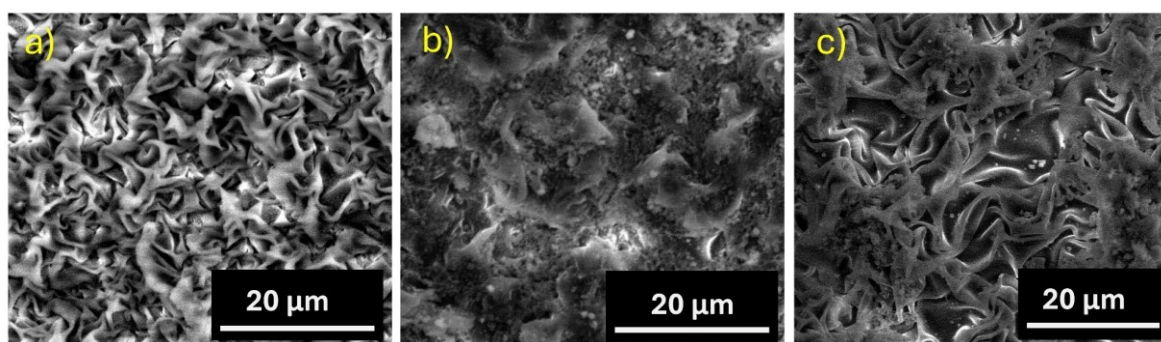


Figure 41 SEM images of wrinkled LNF (a), wrinkled LNF modified with PDA NPs (b), wrinkled HA modified with PDA NPs (c)

Other experiments showed that PDA NPs adhered to LNF wrinkled films, the wrinkles were almost fully covered, which may, again, indicate the beginning of film formation. For future experiments, it might be interesting to explore the process from the beginning of the PDA synthesis to assess the onset of film formation and optimize the conditions for NPs deposition. Then, PDA NPs were observed on wrinkled HA films (picture c). This proved that PDA adheres to both PDA and HA.

It should be noted that some images may have poorer resolution or quality, because the samples were not sputter-coated and the images were acquired under low vacuum using low voltage.

6.4 Ex-situ polydopamine deposition on LNF-HA surfaces

The samples with spin-coated LNF-HA layers in total of 4 or 5 layers were further modified with ex-situ prepared PDA suspension with the objective to create another layer on the prepared films, a layer of PDA. Suspension of various PDA aging times was dropped on the films and left to adhere for 2 minutes. The spin-coated samples were immediately thermally wrinkled and the oxidizer was inactivated. The prepared modified surfaces of psPS-LNF-HA-LNF-HA-(LNF)-PDA were imaged with SEM, as shown in Figures 43-45 below.

Prior to the experiment with dopamine, the same experiment was carried out with same same reagent, except for dopamine, to test the effect of the reaction conditions on the LNF-HA films. The SEM images (Figure 42) of samples tested for reaction conditions are presented before those showing PDA modifications.

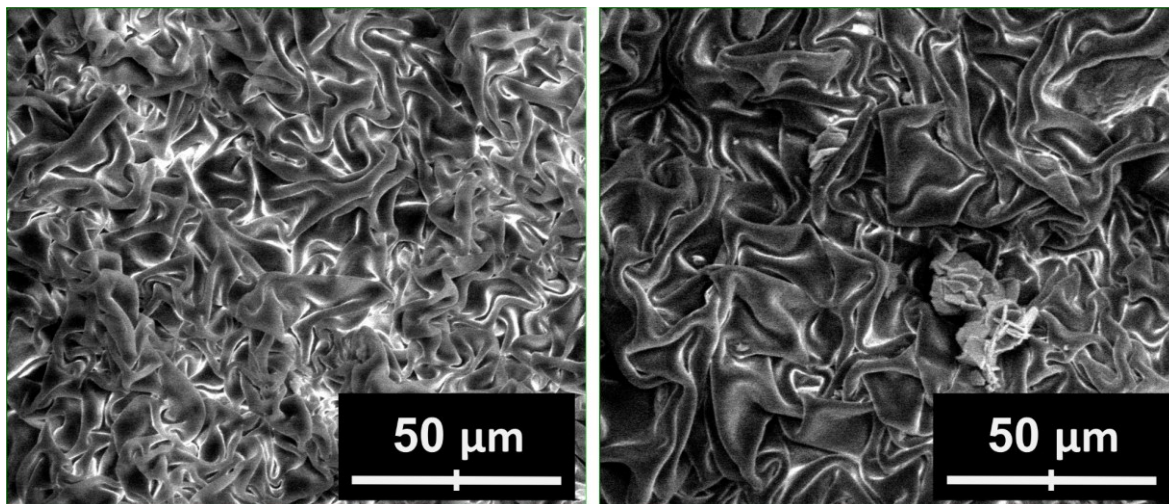


Figure 42 SEM images of wrinkled LNF-HA films (four layer on the left, five layers on the right) exposed to PDA deposition conditions without DA

It was shown that the wrinkled LNF-HA films were not damaged by the reaction conditions, suggesting that this might be a suitable modification method, depending on the other results. A small amount of plate-like structures were observed on the surfaces, probably caused by the crystallization of the salts contained in the solution.

The deposition after 1, 5, 30, 60 minutes and 22 hours of PDA aging time (reaction time) was imaged as shown in the Figures below.

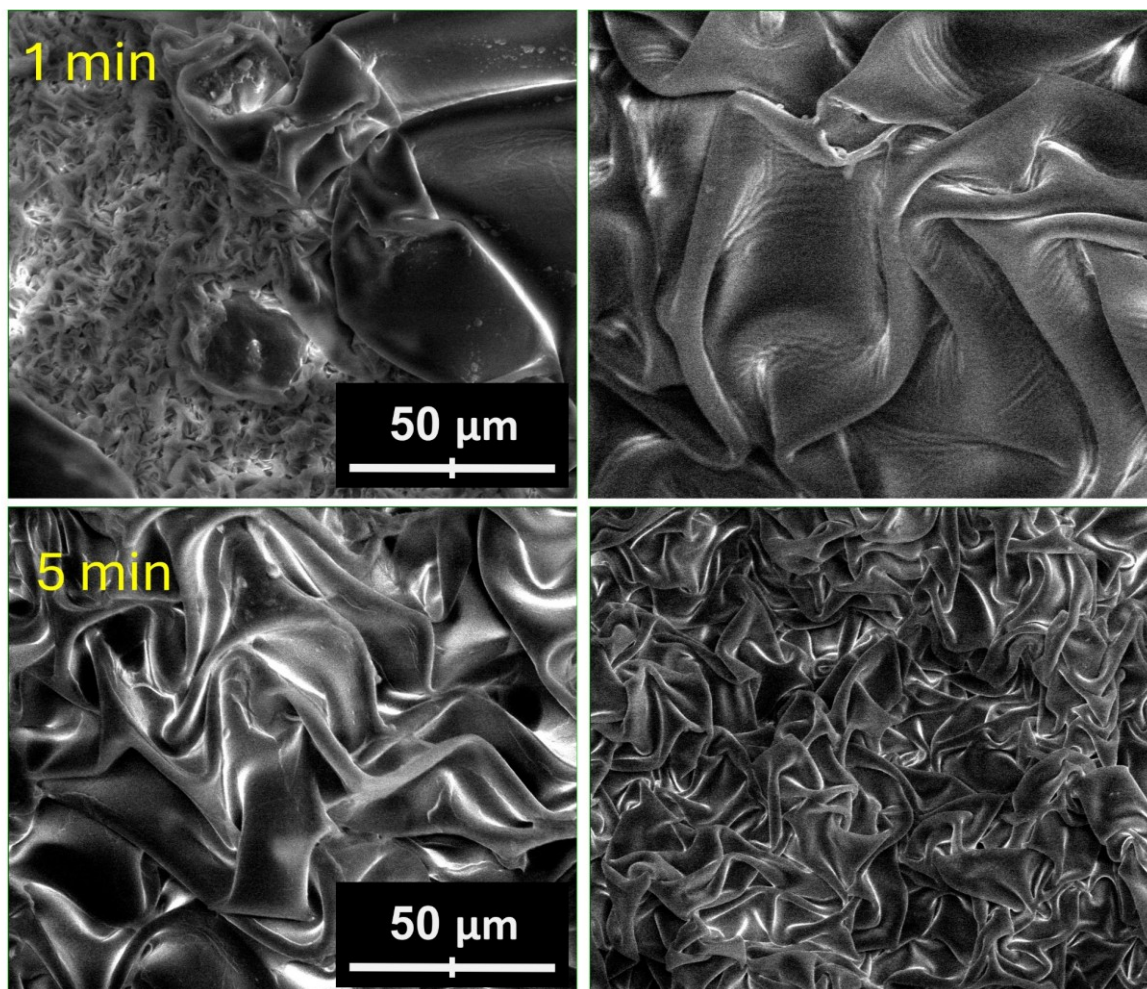


Figure 43 SEM images of LNF-HA films (4L on the left, 5L on the right) modified with ex-situ synthesized PDA aged for 1 and 5 minutes

Low aging times did not result in high PDA coverage. However, some PDA particles can be observed on the surfaces.

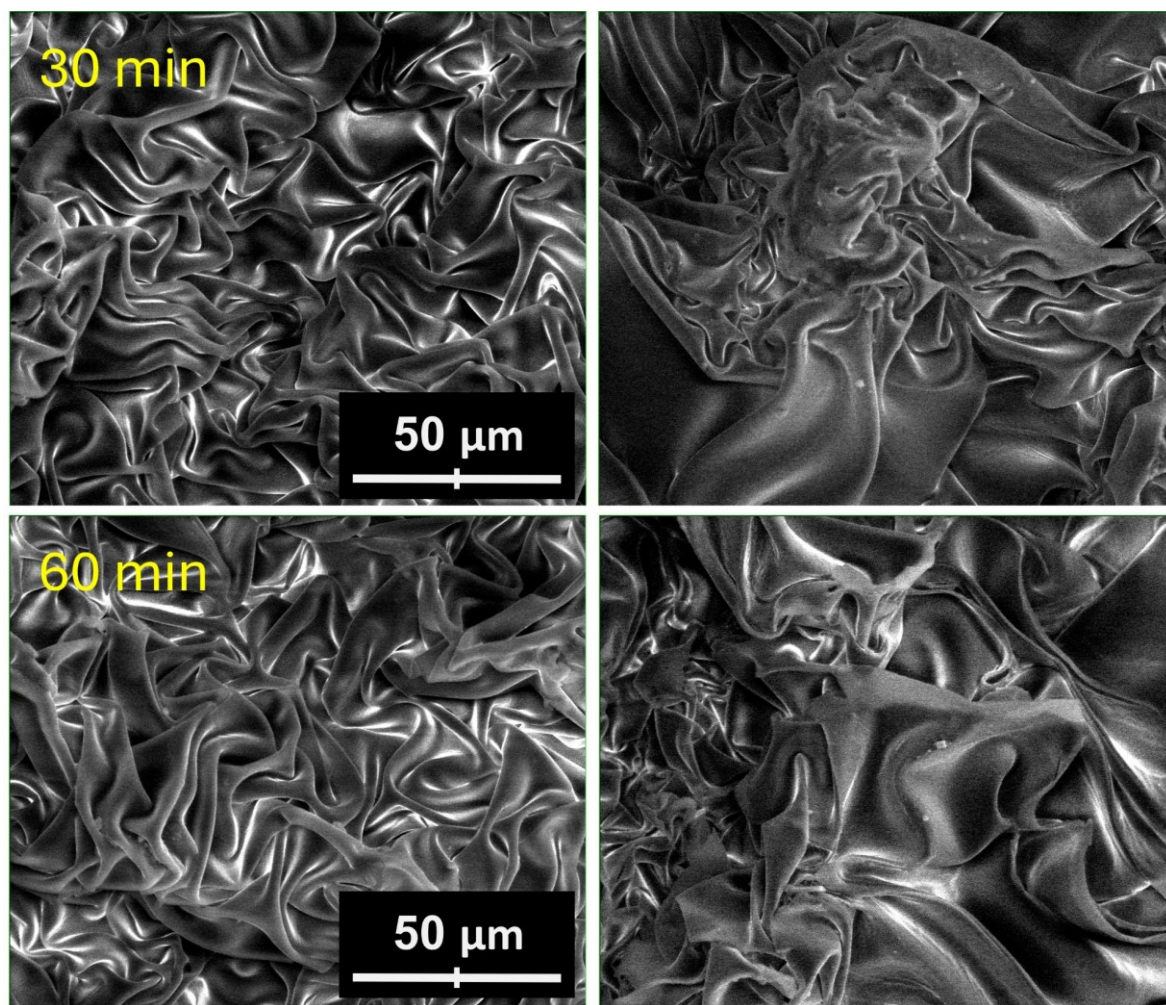


Figure 44 SEM images of LNF-HA films (4L on the left, 5L on the right) modified with ex-situ synthesized PDA aged for 30 and 60 minutes

After 30 and 60 minutes of aging time, more PDA can be observed in SEM images. The coverage is still rather small.

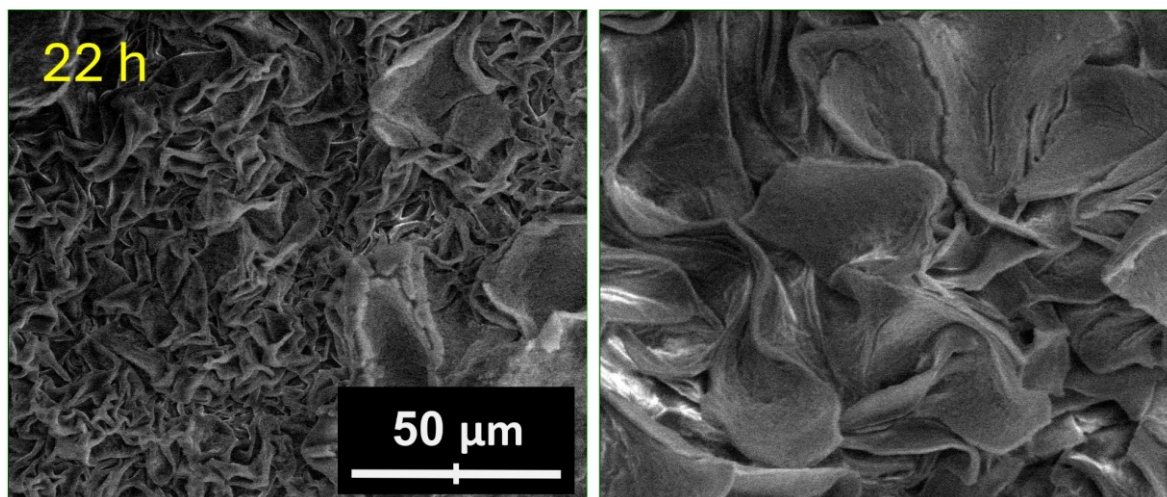


Figure 45 SEM images of LNF-HA films (4L on the left, 5L on the right) modified with ex-situ synthesized PDA aged for 22 h

Despite the expected fast film formation, the film was not observed during the first 60 minutes of the polymerization. After 22 hours, the film was observed in SEM images, exhibiting wrinkled scaly morphology.

6.4.1 Size of PDA particles at different aging times

As previously mentioned, the average particle size of PDA suspensions was determined using dynamic light scattering (DLS). The particle size analysis results, summarized in Table 2, indicate that PDA nanoparticles generally exhibited a narrow size distribution, indicative of a monodisperse system, except for the PDA 1 min sample, which showed a bimodal particle size distribution.

Table 2 Average polydopamine NPs size and polydispersity index measured by DLS at various aging times

Sample	z-average (nm)	Peak1 (nm)	Peak2(nm)	PDI
PDA_1min	790±99	744±47	5375	0.359±0.04
PDA_5min	615±123	469±86		0.391±0.02
PDA_30min	354±130	320±75		0.265±0.09
PDA_60min	150	115		0.319
PDA_20h	3587±932	2403±384	5468±0	0.306±0.03
PDA_22h	2669±190	2472±219		0.213±0.04

Over the course of the reaction, a significant decrease in average particle size was observed, from an initial value of 790 ± 99 nm to 150 ± 0 nm. However, in the sample taken

after 20 hours of reaction, the average particle size increased sharply to 3587 ± 384 nm, exceeding the instrument's detection limit ($>1 \mu\text{m}$).

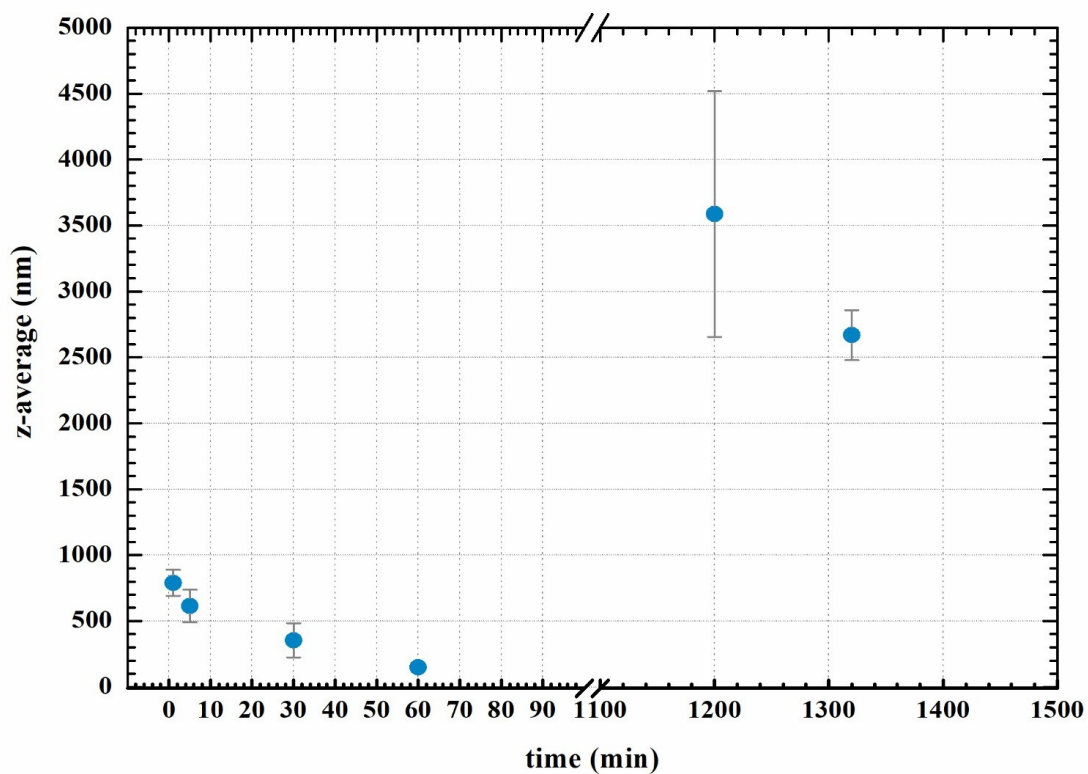


Figure 46 Size measurement of PDA particles at different aging times

This increase is likely due to the aggregation of PDA nanoparticles. As previously discussed, the tendency of nanoparticles to agglomerate depends on the surface charge in the case of solid particles, and in liquid suspensions, primarily on surface morphology and functionalization. (110)

CONCLUSION

To summarize the initial set of experiments, psPS was successfully modified to form wrinkles and PDA NPs were deposited on its surface. The subsequent experiments focused on merging these steps. The wrinkling was carried out either prior to or after the PDA deposition in solution of *Reaction A* (containing ethanol, water, and ammonia to achieve a basic pH)

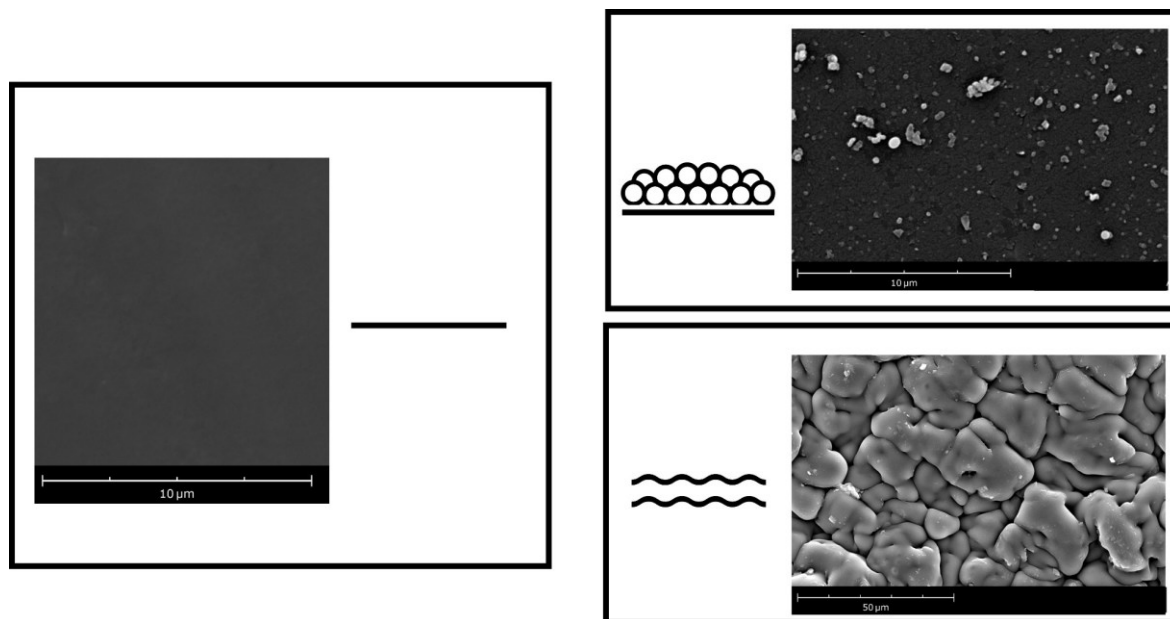


Figure Schematic summary of flat psPS surface modification by PDA NPs deposition (top) and wrinkling (bottom)

It was demonstrated that PDA nanoparticles (NPs) can be deposited onto both flat and wrinkled psPS substrates by immersing them in an alkaline reaction mixture containing water, ethanol, and ammonia. Moreover, the surfaces could be thermally wrinkled without removing the previously deposited PDA NPs.

The wrinkle structure was not affected by the reaction conditions for PDA synthesis. It is likely that if left immersed for more than 22 hours, the PDA starts to form a film, which also wrinkles and detaches from the surface. Furthermore, a potentially interesting, although cost-inefficient, method for pore formation was proposed. This, however, would require further research to confirm the cause and mechanism of the pore formation.

Pre-stressed polystyrene foils were activated with plasma to gain a negative surface charge and used as substrates for film deposition. Lysozyme nanofibers (LNF) suspension with a positive charge and hyaluronic acid (HA) solution with a negative charge were spin-

coated onto the psPS in alternating layers (4 or 5 in total) to achieve good interfacial adhesion necessary for the wrinkle formation by thermal shrinking. The spin-coated films were then successfully thermally wrinkled, as shown by SEM images. The wrinkled samples with the aforementioned variants of spin-coated films and a sample with spin-coated LNF film were then analyzed with FTIR to assess differences between films with either LNF or HA as the top layer, with HA concentrations of 0.5 wt% or 2 wt%, and films composed only of LNF. The LNF concentration was 2 wt% in all samples. The spectra indicated difference in chemical composition of the prepared samples, although some of the absorption bands were the same, probably due to IR beam penetration through the top layer, the samples with HA as a top layer were distinguished by absorption bands in the $950 - 1200\text{ cm}^{-1}$ region, which could be attributed to polysaccharides.

Lysozyme nanofibers were characterized with DLS and circular dichroism. Their hydrodynamic diameter was measured as $244 \pm 16\text{ nm}$ and their zeta potential was measured as $40 \pm 2\text{ mV}$ at $\text{pH} = 2$. Furthermore, CD confirmed the β -sheet structure typical for protein amyloids.

PDA NPs in-situ deposition (same as described previously) was carried out onto psPS substrates with spin-coated films of alternating LNF and HA layers in a total number of four or five layers. The immersion time was 22 hours based on experiments with psPS. SEM images showed successful deposition and only a slight indication of wrinkle disruption. However, the particles adhered inhomogeneously in most areas of the wrinkled surfaces. It was found that in situ deposition is only feasible after wrinkling; if performed beforehand, the films dissolve in the polymerization solution. Both LNF and HA are able to form wrinkles when spin-coated (in four layers) on their own, and PDA NPs are able to adhere to both of these structures.

Finally, ex-situ deposition of PDA was carried out onto psPS substrates with spin-coated films of alternating LNF and HA layers in a total number of four or five layers. The objective of this experiment was to prepare a PDA film and wrinkle it together with previously prepared LNF-HA films. The reaction mixture contained a strong oxidizing agent to speed up the reaction (as PDA polymerization is an oxidation process), and a buffer to maintain a stable pH value of 6. The PDA suspension was spin-coated after 1, 5, 30, 60 minutes and 22 hours of PDA aging time (reaction time) and 2 minutes of adhesion time. The biggest coverage was achieved with 22 hours of aging time, as shown by SEM images, PDA formed a film and wrinkled into flake- or petal-like structures.

Throughout the ex-situ reaction, PDA particle size was measured with DLS. The hydrodynamic diameter of the polymerizing particles decreased during the first 60 minutes from 790 ± 99 nm to 150 ± 0 nm. After 20 hours of reaction, the measured size increased extremely to 3587 ± 384 nm, which exceeds the instrument's detection limit of 1 μ m. This is indicative of particle aggregation, which is consistent with both theoretical and practical observations in PDA polymerization.

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LIST OF ABBREVIATIONS

psPS	Pre-stressed polystyrene
PDA	Polydopamine
PDA NPs	Polydopamine nanoparticles
LNF	Lysozyme nanofibers
HA	Hyaluronic acid
SEM	Scanning Electron Microscopy
FTIR	Fourier Transform Infrared Spectroscopy
ATR	Attenuated Total Reflectance
DLS	Dynamic Light Scattering
CD	Circular Dichroism
PDI	Polydispersity Index
RH	Hydrodynamic radius
wt%	Weight percent
UV–Vis	Ultraviolet–Visible Spectroscopy
SE	Secondary Electrons
BSE	Backscattered Electrons
NP(s)	Nanoparticle(s)