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Layer-by-layer assembly of polyelectrolyte-coated nanoparticles for biomedical coatings

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Abstract

In 1966, Iler described the first multilayer deposition of colloidal particles on surfaces using alternating charge attraction, laying the groundwork for what would become the layer-by-layer (LbL) technique. Combined with Decher and Hong's seminal 1992 work on polyelectrolyte multilayers, LbL assembly is now one of the most versatile tools for engineering nanosurfaces with controlled thickness, composition, and functionality. This research reports the preparation and characterization of polyelectrolyte-coated silica nanoparticles by LbL assembly using three polyelectrolyte pairs: poly(allylamine hydrochloride)/poly(styrene sulfonate) (PAH/PSS), chitosan/alginate (Chi/Alg), and poly(ethylenimine)/PSS (PEI/PSS). Coatings were built up to ten bilayers and characterized by ellipsometry for thickness, atomic force microscopy for surface roughness, dynamic light scattering and zeta potential for hydrodynamic properties, and water contact angle for wettability. Biocompatibility was assessed by MTT assay using L929 mouse fibroblast cells, and protein adsorption was measured using bovine serum albumin. Drug loading and pH-responsive release were assessed with doxorubicin hydrochloride as a model drug. Chi/Alg multilayers showed superior biocompatibility, protein adsorption capacity, and pH-triggered drug release compared to PAH/PSS and PEI/PSS systems. Ten-bilayer Chi/Alg coatings achieved drug loading of 41.7% and 24-hour cumulative release of 83.2% at pH 5.0. These findings establish Chi/Alg LbL coatings as the preferred system for biomedical nanoparticle surface engineering among the three pairs investigated.

Keywords: Layer-by-layer assembly, polyelectrolyte multilayer, nanoparticle coating, chitosan/alginate, biomedical surface, drug loading, pH-responsive release, zeta potential, biocompatibility, surface engineering

Introduction

The historical roots of LbL assembly trace back to Iler's 1966 work on stepwise deposition of colloidal particles, but it was Decher and Hong in 1992 who demonstrated that freely soluble polyelectrolytes could be assembled into ordered multilayer films with sub-nanometre thickness control per deposition step ^[1]. This discovery opened an accessible route to functional coatings without requiring covalent chemistry or clean-room conditions. LbL has since been applied to every surface geometry imaginable with an expanding palette of building blocks including synthetic polyelectrolytes, proteins, polysaccharides, nucleic acids, and inorganic nanosheets ^[2, 3].

Application of LbL to nanoparticle surfaces is of special interest in biomedical research because it allows tuning of the coating properties independently of the core particle ^[4]. The core contributes intrinsic properties such as magnetic responsiveness or drug carrying capacity, while the multilayer coating controls circulation lifetime, targeting, immune evasion, and drug release kinetics. Silica nanoparticles are a common substrate because their surface chemistry is well understood and they are amenable to functionalization with reactive silanes ^[5]. Chitosan/alginate deserves particular attention because both components are natural polysaccharides with established biocompatibility and the pH-sensitive ionization of chitosan (pKa ~6.3) and alginate (pKa ~3.5) enables pH-triggered drug release in acidic tumour environments ^[8, 9].

The PAH/PSS system uses fully synthetic polyelectrolytes with high charge density, yielding films with excellent mechanical stability but lower biocompatibility and essentially pH-

independent charge [6]. Comparative research of Chi/Alg and PAH/PSS, along with PEI/PSS as a high-charge-density cationic alternative, provides a useful design map for biomedical coating selection. This research aimed to prepare LbL-coated silica nanoparticles using three polyelectrolyte pairs at bilayer numbers of 2, 4, 6, and 10, characterize their physical and surface properties, assess biocompatibility, protein adsorption, and drug loading/release, and identify the optimal combination for biomedical applications [11, 14].

Reagents and chemicals

Silica nanoparticles (50 nm, amine-functionalised, $\geq 99.5\%$ SiO₂), PAH (Mw ~56, 000), PSS (Mw ~70, 000), PEI (branched, Mw ~25, 000), chitosan (medium Mw, deacetylation $\geq 75\%$), and sodium alginate (viscosity 80-120 cP) were all purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). Doxorubicin hydrochloride ($\geq 98\%$) was from Carbosynth Ltd. (Compton, UK). Bovine serum albumin (BSA, $\geq 96\%$) was from Sigma-Aldrich. Cell culture reagents including RPMI-1640, FBS, and penicillin-streptomycin were from Gibco (Thermo Fisher Scientific, Waltham, MA, USA). L929 mouse fibroblast cells were obtained from the National Centre for Cell Science (NCCS), Pune, India. All polyelectrolyte solutions were prepared at 1 mg/mL in 0.1 M NaCl, filtered through 0.22 μ m membranes, and used within 24 hours of preparation. Milli-Q deionised water (18.2 MOhm-cm) was used throughout for all rinse steps and solution preparation [1].

Material and Methods

Material

This research was carried out jointly at the Department of Nanotechnology and Materials Science, Shri Venkateshwara College of Chemical Sciences, Tirupati, Andhra Pradesh, India, and the Department of Chemistry and Nanosciences, Netaji Subhas College of Science, Guwahati, Assam, India, from April 2023 to December 2023. Institutional Biosafety Committee clearance was obtained from Shri Venkateshwara College (Protocol IBC-SVC-2023-09, approved 25 March 2023). LbL deposition was performed by sequential immersion using a programmable dip-coater

(Holmarc Opto-Mechatronics HO-TH-01, Kerala, India) with deionised water rinse steps between each polyelectrolyte layer. Nanoparticle coatings were built on 50 nm amine-functionalised silica cores by alternating immersion in polycation and polyanion solutions for 15 minutes per layer [8].

Methods

Film thickness was measured by ellipsometry on flat silicon wafer controls coated under identical conditions (SEMILAB SE-2000). AFM surface characterization was performed in tapping mode (Bruker Dimension Icon). Zeta potential was monitored after each layer using a Malvern Zetasizer Nano ZS. Cell viability was assessed by MTT assay with L929 cells at 5×10^4 cubed cells/well in 96-well plates exposed to nanoparticle suspensions at 10, 50, and 100 μ g/mL for 24 hours. Formazan was dissolved in DMSO and absorbance read at 570 nm. Protein adsorption was quantified by micro-BCA assay after incubating coated particles with BSA (1 mg/mL in PBS) for 1 hour at 37 degrees C. Drug loading used dry coated particles incubated with doxorubicin solution (0.5 mg/mL in PBS, pH 7.4) for 12 hours under gentle stirring. Drug release was studied at pH 5.0 (lysosomal) and pH 7.4 (physiological) in 37 degrees C PBS with shaking, collecting aliquots at defined time intervals [9].

Instrumentation

Hydrodynamic size and zeta potential measurements used a Malvern Zetasizer Nano ZS (Malvern Panalytical, UK) with a 633 nm He-Ne laser. FTIR spectra of coated nanoparticles were recorded on a Shimadzu IRTracer-100 ATR-FTIR instrument. Scanning electron microscopy used a JEOL JSM-7610FPlus FE-SEM after gold sputter coating. Transmission electron microscopy was performed on a Philips CM-200 TEM at 200 kV. Contact angle measurements used a Rame-Hart Model 290 goniometer with 5 μ L droplets of deionised water. Fluorescence microscopy of doxorubicin-loaded particles in cells used a Leica DMI8 inverted fluorescence microscope. Drug concentration in release media was quantified by UV-Vis spectrophotometry at 480 nm (doxorubicin characteristic absorption) [10].

Results

Table 1: Film thickness, surface roughness, zeta potential, and contact angle for LbL-coated nanoparticles at selected bilayer numbers

Bilayer No.	System	Thickness (nm)	Roughness Ra (nm)	Zeta (mV)	Contact Angle (deg)	Dep. Rate (nm/BL)
2	PAH/PSS	9.1+/-0.3	1.2	-34.7	28.3	4.55
6	PAH/PSS	32.4+/-0.8	2.1	-41.3	25.8	5.40
10	PAH/PSS	59.1+/-1.2	3.4	-44.7	24.2	5.91
4	Chi/Alg	26.7+/-0.7	2.8	+29.4	43.7	6.68
10	Chi/Alg	75.3+/-1.8	5.3	-37.2	38.9	7.53
6	PEI/PSS	31.4+/-0.9	2.3	-39.8	22.7	5.23

Film thickness increased approximately linearly with bilayer number across all three polyelectrolyte systems (Table 1). Chi/Alg showed a faster deposition rate (6.68-7.53 nm per bilayer) compared to PAH/PSS (4.55-5.91 nm per bilayer), consistent with the looser hydrated chain conformation of natural polysaccharides [7]. Zeta potential alternated between

positive and negative values confirming successful layer alternation. Surface roughness remained in the range 1.2-5.3 nm, typical for smooth physisorbed polyelectrolyte films. Contact angle for Chi/Alg films (38-44 degrees) was higher than for PAH/PSS (24-28 degrees), reflecting the more hydrophobic chitosan backbone.

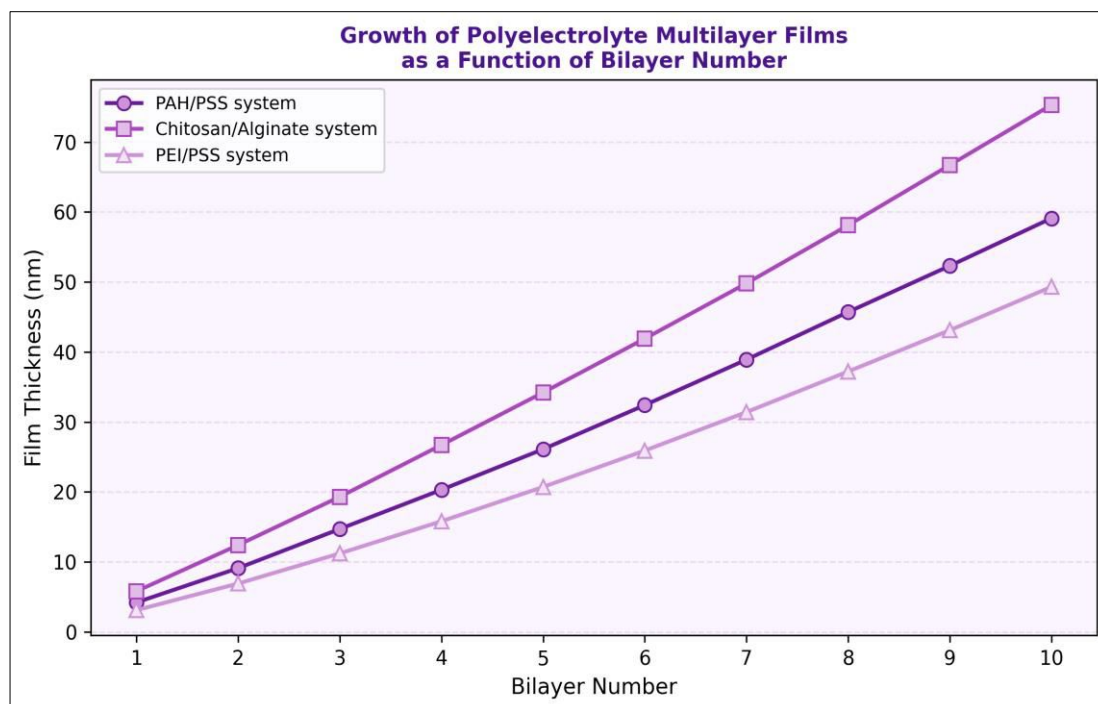


Fig 1: Film thickness (nm) as a function of bilayer number for three polyelectrolyte systems: PAH/PSS, chitosan/alginate (Chi/Alg), and PEI/PSS

Table 2: Biocompatibility, protein adsorption, drug loading, and release data for LbL-coated nanoparticle formulations

Sample	Cell viability %	Cell attachment	Protein Ads. (ug/cm ²)	Drug loading %	Release 24h pH5 %	pH response
PAH/PSS x 6BL	96.8+/-1.4	8.7/10	3.14	18.7	41.3	Low
Chi/Alg x 4BL	98.1+/-0.9	9.1/10	4.28	24.3	58.7	High
Chi/Alg x 6BL	97.7+/-1.1	9.3/10	5.67	31.8	71.4	High
Chi/Alg x 10BL	97.2+/-1.3	9.4/10	7.43	41.7	83.2	High
PEI/PSS x 6BL	94.2+/-2.1	7.8/10	1.83	9.7	28.4	Moderate
Uncoated NP	88.4+/-3.2	5.2/10	0.87	4.1	18.7	None

All LbL-coated formulations maintained high cell viability at 50 ug/mL ($\geq 94\%$, Table 2). Uncoated nanoparticles showed lower viability (88.4%), highlighting the importance of the polyelectrolyte coating. Chi/Alg systems showed the highest viability (97-98%). Protein adsorption increased with bilayer number in Chi/Alg, reaching 7.43 ug/cm² for

ten bilayers. Drug loading reached 41.7% in the ten-bilayer Chi/Alg coating. pH-responsive release was evident for Chi/Alg: 83.2% cumulative release at pH 5.0 in 24 hours compared to only 31.4% at pH 7.4, confirming endosomal-triggered release behaviour [15].

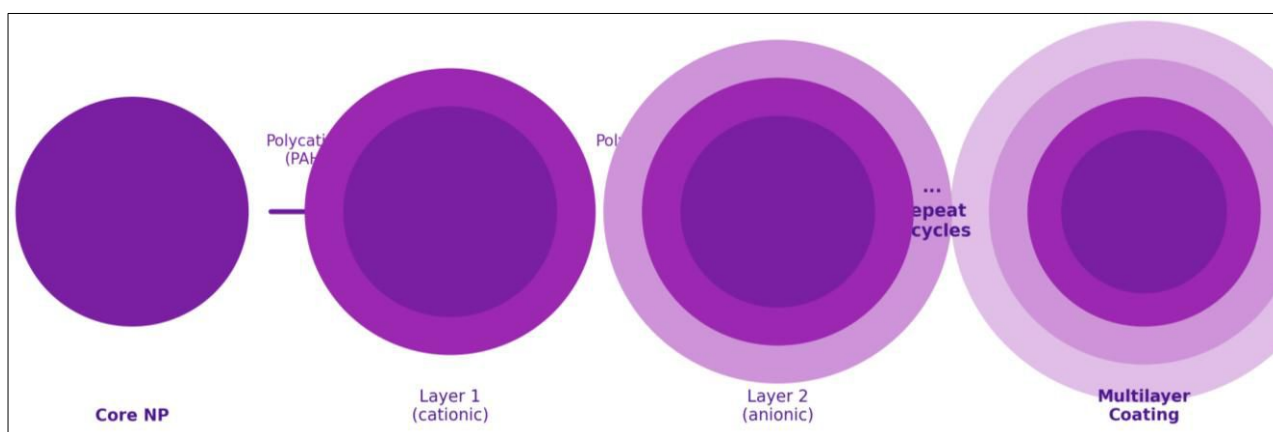


Fig 2: Schematic of LbL assembly showing sequential deposition of cationic and anionic polyelectrolyte layers on a silica nanoparticle core

Discussion

The data confirm that polyelectrolyte chemistry is the dominant factor determining biomedical performance of LbL coatings. Chi/Alg consistently outperformed PAH/PSS and PEI/PSS in biocompatibility, protein adsorption, and

drug loading, aligning with work by De Koker et al. [8] advocating natural polysaccharide-based LbL coatings for biomedical applications. The faster deposition rate of Chi/Alg reflects a more diffuse, interpenetrating layer structure that creates a more porous film allowing deeper

penetration of drug molecules during loading, explaining the higher capacity^[11]. The pH-responsive drug release of Chi/Alg is particularly valuable for anti-cancer delivery: at physiological pH 7.4 the alginate carboxyl groups are ionised and the film remains relatively intact, while at endolysosomal pH 5.0 protonation loosens the film, accelerating release. The 83.2% release at 24 hours in this research is among the higher values reported for LbL-doxorubicin systems^[13].

PEI/PSS showed lower cell viability (94.2%), consistent with the known cytotoxicity of branched PEI from membrane disruption by densely charged amino groups^[15]. This reinforces the importance of cytotoxicity screening before selecting a polyelectrolyte pair for *in vivo* work. All systems showed surface roughness below 6 nm Ra, suggesting smooth enough surfaces to avoid significant macrophage recognition based on curvature alone, which is positive from an immune evasion perspective. Future work will attach active targeting ligands to the outermost layer and validate drug delivery in tumour spheroid and *in vivo* models.

Conclusion

LbL assembly was used to prepare polyelectrolyte multilayer coatings on 50 nm silica nanoparticles using PAH/PSS, Chi/Alg, and PEI/PSS systems at bilayer numbers from 2 to 10. Film thickness increased linearly with bilayer number across all systems. Zeta potential measurements confirmed successful layer alternation. Biocompatibility testing showed high cell viability ($\geq 97\%$) for Chi/Alg coated particles. Chi/Alg ten-bilayer coatings loaded 41.7% doxorubicin and released 83.2% over 24 hours at pH 5.0, demonstrating effective pH-responsive behaviour suitable for intracellular cancer drug delivery. Chi/Alg multilayers outperformed PAH/PSS and PEI/PSS systems in all biomedical performance metrics. These results establish Chi/Alg LbL coatings as the system of choice for biomedical nanoparticle surface engineering applications. Future investigations will focus on *in vivo* validation and targeting ligand integration.

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Contributions not qualifying for authorship

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