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Abstract Template

Novel polyurethanes mimics of antimicrobial peptides

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1.Introduction: Most of the medical devices such as implants, catheters or artificial organs or scaffolds for tissue growth are susceptible to bacterial colonization and prone to formation of biofilms. Adverse potent implications of bacterial colonization include serious chronic infections, which often are life threatening for the patient and are also major cause of rejection of implants. Polyurethanes (PURs) are a large group of polymers widely used in the biomedical and industrial field. Materials with varying chemical, mechanical, biological, and degradation properties can be achieved by proper selection of different reagents (polydiols, diisocyanates and chain extenders). Herein we report the synthesis and characterization of novel PURs mimicking the structure of antimicrobial peptides (AMPs). AMPs are complex biological molecules acts first line of defense in human body against bacterial infection. AMPs are built by short sequences of cationic and hydrophobic amino acids, which imparts amphiphilicity and undergoes self-assembly and self-organization to obtain a unique structural configuration. The unique structural configuration results from distinct segregation of positively charged amino acids and hydrophobic groups onto opposite faces of a helix, sheet, or tertiary structure. Moreover, due to this characteristic they are selective to bacterial cell membrane over mammalian cell membrane. Hydrophobic domains and electrostatic interaction stemming from their amino acid composition, cationic charge and size allow them to interact with the bacterial membrane forming pores and consequently disintegrates the structure. The objective of this work is to synthesize polyurethane based particles by mimicking the structure of antimicrobial peptides so that it is possible to reach a balance between the compatibility of the synthesized polymer to mammalian cells and ability to target desired bacteria.

2.Materials and Methods:

2.a.Synthesis of poloxamer based thermoresponsive amphiphilic polyurethane (PU)
Poloxamer based polyurethane was synthesized by polyaddition process of P407 macrodiol and HDI where N-Boc serinol as chain extender in inert atmosphere using anhydrous 1,2-dichloroethane (DCE) as solvent in the presence of the catalyst (DBTDL)

2.b.Synthesis of polyurethane-g-poly(allyl mercaptan) (PU-SH)
1 g of PU was dissolved in cold water in 3 neck round bottom flask under nitrogen atmosphere. Subsequently, the temperature was raised to 45°C. 5 ml of allyl mercaptan was added with a syringe followed by addition of oxidant ceric ammonium nitrate. The reaction was stopped after 16 hours. The obtained dispersion was purified by dialyzed with distilled water for 3 weeks to remove the water soluble impurities and unreacted thiol and low molecular weight polymer.

2.c.Thiol-ene polymerization of PU-SH and cationic/ zwitterionic monomer (AMPU)
1g of PU-SH was dispersed in water until a homogeneous dispersion was obtained under nitrogen atmosphere and the temperature was raised to 70°C. 5 ml of monomer of interest i.e. (2-Acryloyloxyethyl trimethylammonium chloride or 3-Acrylamidopropyl trimethylammonium chloride or 2-Methacryloyloxyethyl phosphorylcholine) was added with a syringe followed by addition of ammonium persulfate. The reaction was stopped after 16 hours. The obtained dispersion was dialysed with distilled water for 3 weeks to remove unreacted monomer and APS .

2.d.FTIR analysis
Attenuated total reflectance Fourier transform infrared (ATR FTIR)spectra of the polymers were obtained at room temperature in the spectral range from 4000 to 600 cm−1 using a Perkin Elmer (Waltham,MA, USA) Spectrum 100 equipped with an ATR accessory (UATR KRS5) with diamond crystal.

2.e.DLS and Zeta Potential measurement

Diluted solution, 0.01 mg/ml was prepared by dispersing the particles in distilled water (millipore quality). The solutions were stirred for 3 days at room temperature. All samples were filtered using PTFE 1µm disposable filters. All samples were sonicated for 30 minutes prior to the measurement.
The intensity weighted average particle size was determined with a DLS (Zetasizer Nano S90, Malvern Instruments, Worcestershire, UK) at temperatures range from 25° C to 60° C. Zeta potential measurements were carried out on a Malvern Nano ZS 900 instrument.

2.f.*In vitro* cytotoxicity testing: MTT assay

For evaluation of mammalian cell cytotoxicity, NiH3T3 fibroblasts and HaCat keratinocytes were seeded at 10^4 cells/well on the AMPU films. After 24, 48 and 72 h of incubation, MTT (0.5 mg/mL) was added to each well and plates were incubated for an additional 3 h. Optical density (OD) was read at 660 and 570 nm in a microplate reader to evaluate cell viability.

3.Results and Discussion:

Water borne dispersions of thermoresponsive amphiphilic polyurethane grafted poly (allyl mercaptan) were synthesized by aqueous heterophase redox polymerization with ceric ammonium nitrate as oxidant. An aliphatic diisocyante was selected because of negligible toxicity of the biodegradation products of its urethane derivate. Further, synthesized particles were modified with various cationic and zwitterionic monomers like 2-(acryloyloxy)ethyl]trimethylammonium chloride, (3-acrylamidopropyl)trimethylammonium chloride solution and 2-methacryloyloxyethyl phosphorylcholine via thiol-ene polymerizations in aqueous phase in presence of ammonium persulfate. The successful synthesis of polyurethane was confirmed by FT-IR, Raman and NMR analysis. The Zeta potential of particles was also determined. The change in the hydrodynamic diameter of the particles was evaluated by DLS between the temperature interval 15°C- 60°C. Furthermore, the structure of the synthesized particles in dry state was elucidated from TEM micro-graphs. The PUR biocompatibility evaluation using fibroblast and keratinocyte cell line and MTT test is under development.

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