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Mechanically tuned nanocomposite coating on titanium metal with integrated properties of biofilm inhibition, cell proliferation, and sustained drug delivery

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Abstract

The clinical success of coated implants in executing biological functions inclusive of sustainable drug release and long term antibacterial activity without antibiotics is critical. To this aim, a nanohybrid of silver nanoparticles (AgNPs) cored in polyvinyl alcohol nanocapsules (Ag-PVA NCs) embedded in chitosan (CS) matrix loaded with anti-inflammatory drug naproxen was prepared. The synthesized nanohybrids that were subjected to coatings on (3-aminopropyl)triethoxysilane (APTES) treated titanium (Ti) metal exhibited dual role of excellent inhibition on biofilm formation and sustained drug release. These dual characteristics are achieved mainly based on intrinsic antibacterial property of AgNPs and differential entrapment of drug in PVA polymeric shell of AgNPs and CS matrix. The coatings also demonstrated enhanced mechanical properties with increasing inorganic filler and stress shielding on Ti metal. The biocompatibility tests involving adhesion, proliferation and differentiation of osteoblast cells demonstrated the efficacy of Ag-PVA NCs embedded in CS matrix as a suitable coating material for orthopedic applications.

Key words: Microwave synthesis; Silver nanoparticles; Nanohybrid; Chitosan; Nanoindentation; Naproxen

Titanium (Ti) and its alloys are widely used as orthopedic and dental implants due to its excellent chemical stability, mechanical and biocompatible features.[1] However, the drawbacks of poor bone cell adhesion and low proliferation ability restricted the usage of Ti implants for diseased part.[2] To mitigate this problem, efforts were devoted toward bioactive coatings/functionalization on Ti surface by using calcium phosphates, biomolecules, polymers and peptides.[3] These modification procedures gained least attention due to their incompetence to counter bacterial infections and inflammations at the implant site. Recent reports demonstrated that the accumulation of physiological reactive oxygen species (ROS) tends to alter the signaling pathways and results in the poor interaction between implant and existing bone.[2,4] Sustainable anti-inflammatory drug releasing coatings could be a suitable approach to sort out complications related to inflammation. Nevertheless, biofilm formation that is associated with bacterial adhesion and its subsequent colonization at the implant site is also considered a major cause for implant failures.[5] The conventional antibacterial therapies are effective against systemic infections but with seldom success against biofilms.[6,7] Moreover, attempt to treat biofilm via conventional therapies is expected to enhance patient complications due to the usage of high dose antibiotics.[7,8] These biofilms enable bacteria to develop resistance against antibiotics and immune responses.[9,10] Unfortunately, the lack of options for a proper medical treatment at the implant site is left with no option other than its removal.[11]

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With the outbreaks of multiple-drug resistant (MDR) and pan-drug-resistant (PDR) pathogenic[12] bacteria, current attention is emphasized on non-antibiotic nanomaterials which have intrinsic antibacterial properties.[13] Advancement in nanotechnology has resulted in the use of various metal/metal oxide nanoparticles as effective antimicrobial agents at physiological conditions.[14,15] Silver nanoparticles are proven as potent broad spectrum antimicrobial agents that deliver protection against both Gram positive and negative bacteria.[16,17] Various synthetic methods are reported for the synthesis of silver nanoparticles (AgNPs) such as photoreduction, electron beam, γ-irradiation and use of chemicals as strong reducing agents.[2] The antibacterial competence depends on size, shape, nature and stabilizer of nanoparticles and its ability for homogenous dispersion in the polymer matrix to counter biofilm formation.[16,18,19] In addition, the use of harmful reducing agents and organic solvent in the synthesis process is not preferred owing to the concern for carcinogenic and environmental issues.[19]

Recently, chitosan (CS) has found applications in wound dressings, tissue engineering scaffolds and implant coatings due to its exceptional biocompatible features.[20,21] Li *et al*. reported improved osteointegration on CS coated porous Ti implants.[2] However, the disadvantages of fragility, poor mechanical stability, fast oxidative and enzymatic degradation restrict CS as a source for coating on metallic implants. This limitation could be rectified by blending CS with other natural or synthetic polymers to achieve a superior stable coating.[22] Poly vinyl alcohol (PVA), a water soluble polyol, is a highly compatible biodegradable polymer due to its strong tendency to form intermolecular hydrogen bonding with biopolymers.[23]

Previously, the authors reported on the tunable mechanical properties of coatings with varying concentrations of CS and PVA blend on Ti metal.[24] Moreover, CS and PVA blend possessed the ability to shield shear stress of Ti metal.[24] Our recent report on AgNPs embedded in PVA-CS coating on Ti implants demonstrated good mechanical stability along with excellent antibacterial features.[25] The particular study also emphasized the safety method of AgNP synthesis through polyol reduction method using PVA as reducing as well as capping agent.

In present study, AgNPs cored in PVA nanocapsules were synthesized through microwave irradiation as a system to hold the drug in core for sustained release and its uniform distribution in CS matrix, which is another drug loading platform for initial burst release. Hence, varying concentrations of AgNPs cored in PVA nanocapsules have been compared in terms of mechanical strength, biofilm inhibition ability and dual tuned drug releasing ability. Naproxen, an anti-inflammatory drug was selected as a model drug and sustainable drug release was achieved in PBS solution. The cell viability, adhesion and proliferation ability were tested in Saos-2 cells.

Methods

"Green synthesis" of AgNPs cored in PVA nanocapsules (Ag-PVA NCs)

Ag-PVA NCs were synthesized according to our previous report.[25] Briefly, 10 mM $AgNO_3$ was added to the 5 mg/ml PVA solution at a constant pH maintained in the range of 8.5 ± 0.3. This solution mixture was then irradiated in microwave oven for 4-5 min at a constant power of 300 W until the solution color turned golden yellow. This was followed by cooling the solution in ice bath and subsequent centrifugation at 16,000 rpm for 30 min to collect AgNPs cored in PVA nanocapsules.

Synthesis of Ag-PVA NCs embedded in CS composite systems

Aqueous CS was prepared by dissolving known amount of CS in 1% acetic acid solution to make the final concentration of CS as 6 mg/ml. Ag-PVA NCs embedded in CS composite were prepared by a simple approach of mixing known weight of Ag-PVA NCs in 5 mg/ml CS solution. Three different concentrations of Ag-PVA NCs were selected as 1 mg/ml, 5 mg/ml and 10 mg/ml, denoted as Ag 1, Ag 5 and Ag 10, respectively. Samples were vigorously stirred at room temperature overnight. Drug loaded samples were synthesized by adding known weight of naproxen during the synthesis to make final concentration of drug as 10 mg/ml.

Fabrication of nanohybrid coatings

Nanohybrid solution (100 μL) was homogeneously spread onto the pretreated Ti substrates (1 cm^2) and dried at room temperature in a vacuum oven for 24 h. For growth inhibition assay, Ti specimens were coated on both sides similarly as mentioned above. The dry coated Ti substrates were stabilized in 2 N NaOH for 5 min and washed in deionized water for further characterization.

Characterizations

Characterization techniques and methods are conferred in supplementary information.

Results

Characterization of AgNPs in cored PVA nanocapsules (Ag-PVA NCs)

In order to confirm the AgNP synthesis, UV–vis absorption spectra were explicated. The SPR peak at 410 nm observed for Ag-PVA NC solution (Figure 1, *A*) confirms the successful synthesis of AgNPs. To elucidate the crystalline nature and phase purity, XRD analysis was performed for powdered Ag-PVA NC samples (Figure 1, *B*). The face-centered cubic (FCC) nature of Ag is confirmed from the observed XRD reflections that showed excellent matches with the standard JCPDS card no. 65-2871 of Ag.

The size and morphology of AgNPs and Ag-PVA NCs were determined from TEM. The high resolution TEM image of Ag-PVA NCs confirms the spherical AgNPs in PVA capsules (insets of Figure 1, *C*). Figure 1, *C* also confirms the nearly spherical nature of PVA in the form of capsule that tends to accommodate AgNPs in its core matrix. It should be noted from Figure 1, *C* that larger capsules of PVA possess more than one AgNP in its core. Hydrodynamic size (D_H) of Ag-PVA NCs measured from DLS indicated an average D_H of 113 nm with polydispersity index <0.3 (Figure S1). The selected area diffraction pattern (SAED) revealed polycrystalline nature with well reflected rings that correspond to the *d*-spacing of Ag (Figure 1, *D*).