RESEARCH PAPER

Optical and Biological Characterization of Smart Polymer Based Carriers for Nano-Gold and Allogenic Platelet Rich Plasma for Dental Tissue Regeneration

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ABSTRACT

Gold Nanoparticles (AuNPs) are used in a variety of biomaterials to amplify the bioactivity. In the present study, the characteristics of allogenic human Umbilical Cord Blood-Platelet Rich Plasma (UCB-PRP) conjugated with gold nanoparticles and smart polymer was assessed. Gold nanoparticles-15nm (5ml) is centrifuged to avoid any gold aggregation. About 1mg of lyophilised UCB-PRP is added and incubated for 12hrs at room temperature. Smart polymer Poly-(N isopropylacrylamide) (PNIPAM) was separately added to AuNPs and UCB-PRP solution and stirred to obtain Smart polymer-AuNP-UBC-PRP conjugate. The optical properties and the overall stability of the drug is assessed using UV-Vis spectroscopy, Polarize Light Microscopy and Zeta potential along with antibacterial and cytotoxicity assay. The UV–Vis absorption of Smart polymer-AuNP-UBC-PRP showed PNIPAM characteristic at 240nm. PLM-Dark field images indicated the relative position of individual particles. Zeta potential obtained was +14.71 mV. AuNPs showed efficient antibacterial property against Streptococcus mutans. The cytotoxicity assay showed that the conjugate maintains high cell viability (>94%) in HDPSCs across all tested concentrations indicating low cytotoxicity.

Keywords: Smart polymer; Poly (N-isopropylacrylamide); Gold nanoparticles; Umbilical Cord Blood Platelet-Rich Plasma.

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1. Introduction

Nanotechnology has advanced significantly in the last few decades [1]. The potential of nanomaterials to improve disease prevention, diagnosis and treatment has generated a lot of interest. Nanomaterials are defined as substances made up of structural units, particles, fibers or grains that are less than 100 nm in at least one dimension. Their remarkable physicochemical qualities and biomimetic traits play a crucial role in directing tissue regeneration and encouraging cell proliferation [2].

Gold nanoparticles (AuNPs) are generally considered far less toxic than many other nanoparticle types, offering promising prospects for future applications. They are biocompatible, readily conjugated with biomolecules and hold significant potential for biomedical uses such as imaging, diagnostics and therapeutic interventions for

cancer and various other human diseases [3]. AuNPs, due to their nanosize, have larger surface area, which allows for more interaction with inorganic and organic molecules. AuNPs due to its good biocompatibility and surface specificity can be used as osteogenic agents for bone regeneration [4]. Some studies have shown the effectiveness and safety of chitosan–gold nanoparticle–peroxisome proliferator-activated receptor gamma (PPARγ) conjugates in preventing inflammation and promoting osteoblast proliferation on titanium implant surfaces [4,5]. AuNPs have been studied for its effects on stems cells in tissue engineering evaluated application of AuNPs on cell matrix adhesions and its effect on behaviour of cell morphology [6]. A protuberant Surface Plasmon Resonance (SPR) feature of AuNPs, combined with their tunable size and shape, size-dependent optical properties and ease of functionalization make AuNPs promising and versatile tools for applications in tissue engineering and regenerative medicine [7]. Owing to their biocompatibility, high surface area and antimicrobial properties, gold nanoparticles are widely explored in dentistry for therapies ranging from periodontal treatment to restorative biomaterials and tissue regeneration. AuNPs enhance material strength, offer therapeutic benefits across various sizes and concentrations and serve as effective fillers in restorative biomaterials [8].

Umbilical Cord Blood Platelet-Rich Plasma (UCB-PRP) serves as an effective alternative to both autologous adult blood PRP, gaining recognition for its high concentration of growth factors [9]. The residual blood in the placenta and umbilical cord is collected after delivery to obtain UCB-PRP which contains key growth factors such as Platelet-derived growth factor (PDGF), which promotes cell chemotaxis and Transforming growth factor-beta (TGF-β) which stimulates extracellular matrix synthesis. The existence of growth factor Vascular endothelial growth factor (VEGF) helps in endothelial cell proliferation and angiogenesis. Also, Fibroblast growth factors (FGF), which drives fibroblast migration, proliferation and angiogenesis and Insulin-like growth factor 1 (IGF-1), which enhances cell proliferation and prevents apoptosis. The presence of Epidermal Growth Factor (EGF) aids epidermal fibroblast proliferation and keratinocyte migration [10].

Poly(N-isopropylacrylamide) (PNIPAM) is a well-known temperature-sensitive polymer with a low critical solution temperature (LCST) of around 32 °C, close to body temperature. This property makes it highly suitable for pharmaceutical applications, including nanocarrier-based drug delivery and thermogels. When incorporated into nanocarriers, PNIPAM can improve drug delivery efficiency and enable sustained release, reducing the risk of burst drug release in temperature-responsive hydrogel systems [11]. PNIPAM, characterized by its amide and isopropyl groups in the monomer structure, is typically synthesized by crosslinking PNIPAM or its derivatives. It undergoes a sharp and reversible volume phase transition, swelling or shrinking near its LCST. This transition not only alters particle size but also affects other properties, including hydrophilicity, transparency and apparent electrostatic permittivity [12].

There is a growing need for advanced biomaterials that can support regeneration in dental tissues while being biocompatible, stable and therapeutically effective. Thus AuNPs, due to their unique optical and antibacterial properties may offer promising potential but require stabilization when combined with allogenic UCB-PRP. A smart polymer like PNIPAM can support controlled delivery of AuNP-UCB-PRP to the target tissue. The aim of the study is to develop and assess the optical stability, antibacterial efficacy and biocompatibility of the conjugated PNIPAM and AuNPs-UCB-PRP.

2. METHODOLOGY

2.1 *Preparation of Lyophilized UCB-PRP*

Fresh full-term Human Umbilical Cord blood (40 weeks of gestation) within 30 minutes of delivery was obtained. Cord blood was collected using 50 ml sterile vacutainer with anticoagulant (Ethylene Diamine Tetra-acetate). It is immediately subjected for centrifugation of 1400 rpm for 10 minutes and the buffy coat thus formed is transferred to another tube for further centrifugation of 2400 rpm for 15 minutes. The accumulated PRP is separated and pooled samples will be pre-frozen at -80˚C and lyophilized using freeze drying method [10,13].

2.2 *Synthesis of Smart polymer-AuNPs-UBC-PRP*

Gold Nanoparticles (25ml) (15nm, obtained from Sigma Aldrich, product number 777137) were centrifuged at 500 rpm for 15 minutes to prevent gold aggregation and ensure uniformity. Subsequently, 5 microliters of lyophilized PRP were added and incubated for 12 hours at room temperature. PNIPAM (5 micrograms, procured from Thermo Fisher, product number AC412780250SDS) was separately combined with 5 microliters of AuNPs. Following this, both were mixed and stirred to achieve a homogeneous blend and then centrifuged at 10000 rpm to obtain the final mixture.

2.3 *Characterization of Smart polymer-AuNPs-UBC-*

PRP

2.3a *UV-vis absorption spectra*

The optical properties and the overall stability of the drug were assessed using UV-Vis spectroscopy. Quartz crystal cuvettes with a path length of 10 mm were used to obtain adsorption spectra using 2 ml 1% wt. 2 ml drug is analyzed using scan speeds of 400 nm/min to record wavelengths over 1100 to 200 nm.

2.3b *Polarize Light Microscopy (PLM)*

AuNPs imaging was done with the 20 µL prepared solution dropped on the slider slips. The samples were covered by coverslips and then were immediately investigated under PLM. The focus plane was gradually adjusted to find AuNPs. The exposure time was 100 ms. BX 53 Research Microscope with polarizer attached was used.

2.3c Zeta *potential*

The effective surface charge of the drug formulation was determined using zeta potential measurements, which provide insight into colloidal stability and particle–particle interactions. Measurements were performed in aqueous suspension using a dynamic light scattering instrument equipped for electrophoretic mobility analysis. Samples were analyzed under monomodal acquisition and the resulting electrophoretic mobility data were converted to zeta potential values using the Smoluchowski approximation, which assumes a thin double layer relative to particle size.

2.3d *Assessment of antibacterial properties by zone of inhibition*

Streptococcus mutans (ATCC 25175) is used to evaluate the antibacterial activity of the drug by inhibition zone method. It is incubated separately with a liquid culture medium (Mitis Agar), and 200 µL of bacterial suspension (107–108 CFU/mL) was spread on the agar plate. Filter paper discs (1.5mm in diameter) placed on the agar plates and Smart polymer-AuNPs-UBC-PRP (1 %, 25 µL) is dropped on the filter paper discs. After incubation at 37 °C for 12 hrs, the sizes of inhibition zones were recorded.

2.3e *Minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)*

MIC and MBC of drug were evaluated by broth microdilution method. Bacteria suspensions was diluted to around 2×105 CFU/mL with nutrient broth. About 10 µL of the diluted suspension was added into 100 µL of broth containing various concentrations of the drug in a 96-well plate. After incubation at 37 °C for 8 h, the MIC and MBC were calculated.

2.3f *Cytotoxicity assay*

HiFi™ Human Dental Pulp Stem Cells (DPSC) (Product code: CL008) cell viability was evaluated by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay. Incubated 1×10^4 L929 cells per well in 96-well plates with 10–200 µg/mL of samples in 200 µL of DMEM medium for 24 or 48 hrs. 10 µL of MTT solution was added, and 10 µL of dimethylsulfoxide after incubation. The absorbance at 450 nm was recorded by a microplate reader.

3. Results and Discussion

3.1 *UV-visible spectroscopy*

UV-visible spectroscopy provides an accurate and simple method for determining geometry around the transition metal ion in the formation of the polymer complexes. The UV-Vis spectrum exhibited a characteristic absorption peak at 240 nm (Figure.1), indicating the presence of PNIPAM in the Smart polymer-AuNPs-UBC-PRP composite. This confirms the successful incorporation of the temperature-responsive polymer into the hybrid system. A similar study showed UV-visible spectrum of Cu2-doped poly(N-isopropylacrylamide) created a new band position at 276 nm and 278 nm and another study depicted peak located at 200–300 nm in the UV-Vis spectra for AuNPs-PNIPAM conjugate at room temperature [14,15].

3.2 *Polarised light microscopy*

To understand the physical and structural arrangement the drug was observed under PLM (Figure.2). PLM is a scattering-based technique which produces a bright image of the specimen on a dark background. Under polarized light, a distinct birefringent signal in the Smart polymer–AuNPs-UBC-PRP conjugate were observed, confirming semi-crystalline domains. This suggests that AuNPs and protein-polymer interactions have induced localized structural organization, which could be beneficial for mechanical stability or biological performance. The strong scattering signal of AuNPs is attributed to their high scattering coefficients (~5 orders of magnitude higher than conventional fluorescent dyes) [16]. As the nanoparticles scatter light most strongly, some studies showed that the wavelength of the Surface Plasmon Resonance (SPR) was maximum, the nanoparticles appeared in brilliant color that depends on the size and shape of the particles [17].

3.3 *Zeta potential*

Zeta potential reflects the types of ionic species and the number of charges. A zeta potential of +14.71 mV (Table.4), moderate positive charge may be due to PRP proteins or PNIPAM polymer coating on AuNPs. A phase vs. time plot of zeta potential (Figure.3) measurement shows steady, linear slope indicating consistent particle movement under the applied electric field, reflecting stable measurement conditions. The calculated mean zeta potential (+14.71 mV) suggests moderately stable, positively charged nanoparticles with no aggregation was favorable for electrostatic interactions with negatively charged biological membranes. Studies with chitosan showed a zeta potential of 33.8 ± 0.6 mV, reduced to 30.7 ± 0.5 mV in PNIPAM–Chitosan due to partial neutralization of amino groups by uncharged PNIPAM. The retained positive charge favors interaction with negatively charged cell membranes, enhancing cellular uptake and delivery in regenerative applications [18]. In other experiments, the absolute zeta potential of bare AuNPs (−48 mV) was substantially higher than that of AuNPs–PNIPAM (−28 mV), indicating a reduction in surface charge magnitude following polymer coating [19].

3.4 ***Antibacterial property***

The results antimicrobial activity of Smart polymer-AuNPs-UBC-PRP was assessed by disc diffusion method showed antibacterial potency of test compound against bacterial strains tested, *Streptococcus mutans* compared to standard and control used for the study showing a clear zone of inhibition of 25mm (Figure.4 and Figure.5). Studies have demonstrated AuNPs antibacterial activity against both Gram-positive and Gram-negative bacteria, with MIC values around 120 µg/mL. At these concentrations, AuNPs interact directly with bacterial membranes causing penetration and lysis, aided by their high surface-to-volume ratio. Smaller particles (~25 nm) show enhanced uptake and improved antimicrobial effects. Mechanistically, AuNPs inhibit protein synthesis by blocking tRNA–ribosome binding and impair metabolism by altering membrane potential and inhibiting ATP synthase, leading to ATP depletion. As these effects are independent of Reactive Oxygen Species generation, AuNPs exhibit reduced toxicity toward mammalian cells [20,21].

There was significant microbial suppression in MIC assays (Figure.6), indicating concentration-dependent antimicrobial activity and effective bactericidal action at relatively low MBC values(0.25mg/ml) (Figure.7). The MBC was higher in non-visible dilution (0.5 mg/mL) compared to visible dilution (0.25 mg/mL) (Figure.8) indicating that a greater concentration was required to achieve complete bacterial killing. These findings suggest that the synergistic combination of gold nanoparticles and UCB-PRP components holds substantial potential as an antimicrobial agent. The antimicrobial activity where the interaction between the microorganisms and nanoparticles is exploited, due to the size or shape of the nanoparticles, it has ability to make changes the permeability of the microorganisms by making gaps or bore, thus can be inhibiting the enzymatic activity of the respiration leading to apoptosis of the cells. The interaction between SH groups of proteins and AuNP ions plays major role in bacterial inactivation [22,23]. The presence of AuNPs in the electron dense granules observed after AuNP treatment in the cytoplasm of bacterial cells suggests an interaction with nucleic acids that probably results in the impairment of DNA replication [24].

3.5 ***Cytotoxicity assay***

The cytotoxic potential of the Smart polymer–AuNP–UBC-PRP conjugate was evaluated using the MTT assay on HDPSCs across a range of concentrations (Figure.9). The results demonstrated consistently high cell viability (>94%) at all tested concentrations (Table 5), indicating that the conjugate did not induce significant cytotoxic effects at 200µg/ml after the treatment period of 24hrs. The observed results depict the non-toxic potency of compound till 200µg/ml concentration. Microscopic examination of the cultured cells revealed normal spindle-shaped morphology with no observable signs of cell shrinkage, detachment or membrane disruption (Figure.10). The cytotoxicity of PNIPAM and its thiolated conjugate when evaluated in HeLa and MDA-MB-231 cells using the CCK-8 assay after 24 and 48 hours of exposure, exhibited no significant toxicity, even at high concentrations of 500 µg/mL [25]. In both cell lines, AuNPs–microgel composites exhibited no significant cytotoxicity at any concentration when tested. In contrast, some studies showed that bare AuNPs induced dose-dependent cytotoxicity, with MTT assay results showing reduced cell viability to 84%, 78% and 62% at concentrations of 25, 50 and 100 µg/mL, respectively [26].

AuNPs conjugations have been studied with blood components such as plasma proteins, coagulation factors and platelets [27]. Studies on AuNPs with particle sizes of 30 and 50 nm when incubated with blood components and tested for coagulation and platelet aggregation showed no effect on the nanoparticles. Incubation with blood plasma increased the AuNPs surface charge with no influence on the size of the proteins in the blood [28]. Gamma-globulin, albumin and fibrinogen change their conformation when their interactions were studied with

AuNPs of 5–100 nm. When improved with sulphonated chitosan and pyrimidine, AuNPs of 13 nm were verified to have anti-thrombogenicity, suggestive of inhibiting platelet aggregation, interfering with thrombin and fibrin along with prolonging clotting time [29].

The MTT assay results in the present study suggest us that, given test compound, Smart polymer-AuNPs-UBC-PRP was non-cytotoxic on HDPSCS cells at 200µg/ml after the treatment period of 24hrs depicting the non-toxic potency of compound till 200µg/ml concentration. However, further studies must be conducted to determine the molecular mechanism behind cell proliferative properties of the test compound at in-vitro level.
These findings illustrate the possible potential of Smart polymer-AuNPs-UBC-PRP for tissue engineering functions. PRP generated from cord blood also contained greater amounts of VEGF, a key molecule in the promotion of angiogenesis and neovascularization [30]. Thus, Smart polymer-AuNPs-UBC-PRP showed that the properties of UBC-PRP sustained after the addition Smart polymer-AuNPs.

The storage stability and long-term release behavior of the Smart polymer-AuNPs-UBC-PRP requires to be assessed further. Minor batch-to-batch variations in PRP composition or polymer–nanoparticle mixing may influence the reproducibility of the properties of the conjugate, which could become more significant during scale-up or clinical translation. These limitations highlight the need for further in-vivo studies, stability testing and process standardization before potential clinical application in regenerative dentistry.

4. Conclusion

In this study, lyophilized umbilical cord blood PRP was combined AuNP and PNIPAM to form a complex smart biomaterial for potential use in regenerative endodontics. Characterization using UV – visible spectroscopy, Polarized Light Microscopy and zeta potential confirmed retention of polymer properties and successful conjugation along with antibacterial and cytotoxicity assays demonstrating biocompatibility and efficacy of Smart polymer-AuNPs-UBC-PRP. This conjugate shows smart polymer providing a special control for nano–drug delivery, although warranting further *in-vivo* evaluation for clinical translation.

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Optical and Biological Characterization of Smart Polymer Based Carriers for Nano-Gold and Allogenic Platelet Rich Plasma for Den-tal Tissue Regeneration

TABLES

Table 1. Zeta potential of Smart polymer-AuNPs-UCB-PRP

	Mobility	Zeta Potential(mV)	Rel.Residual
Mean	1.15	14.71	0.0687
Std.Error	0.26	3.30	0.0175
Combined	0.87	11.16	0.0468

Table 2. Zone of inhibition by Disc Diffusion Method- Smart polymer-AuNPs-UCB-PRP on S.mutans

	Zone of inhibition (mm)			
Culture condition	Average	SD	SE	ZOI±SD
Control	0	0	0	0
Std control-30ug	27	1.414214	1	27±1.41
SP-Au-UPRP-1mg/ml	25	1.414214	1	25±1.41

Table 3. Minimum Inhibitory Concentration activity-Smart polymer-AuNPs-UCB-PRP -*S.mutans*

Drug conc (mg/ml)	Average	SD	SE	Inhibition±SD (%)
1	99.88	0.065048734	0.0459964	99.88±0.06
0.5	98.06	0.882690651	0.62415655	98.06±0.88
0.25	98.73	0.34199624	0.24182786	98.73±0.34
0.125	92.08	1.044225691	0.73837907	92.08±1.04
0.06	80.15	3.396476994	2.40167191	80.15±3.39
0.03	50.15	0.356469186	0.25206178	50.15±0.35
Positive control	99.69	0.040450028	0.02860249	99.69±0.04
Negative control	0.00	0	0	0

Table 4. Minimal Bactericidal Concentration activity of Smart polymer-AuNPs-UCB-PRP S.mutans

Dilution	Conc (mg/ml)
Visible dilution	0.25

Non-visible dilution	0.5

Table 5. Cytotoxicity assay of Smart polymer-AuNPs-UCB-PRP

Culture condition	% cell viability
Untreated	100.00
Dox-1µM	77.99
SP-Au-UPRP-12.5µg	99.87
SP-Au-UPRP-25µg	99.53
SP-Au-UPRP-50µg	99.06
SP-Au-UPRP-100µg	98.46
SP-Au-UPRP-200µg	94.58

Figures

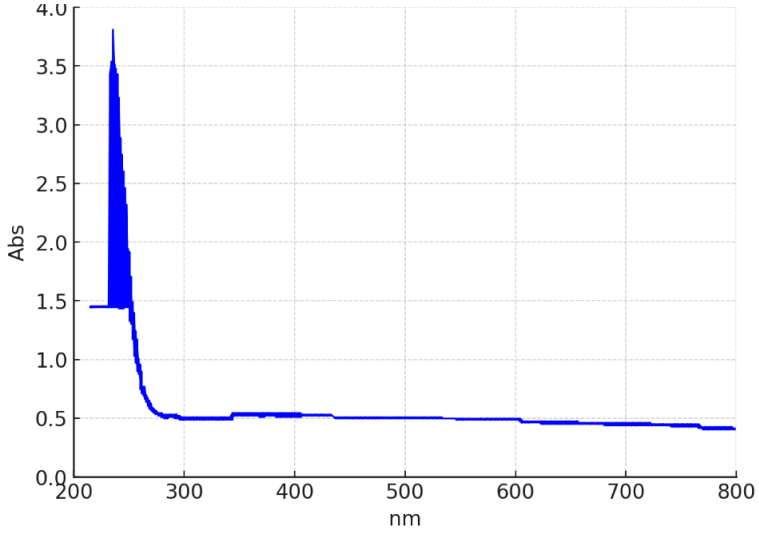

Figure 1.UV – visible absorption spectra of Smart polymer-AuNPs-UCB-PRP shows peak ~240nm corresponding to PNIPAM's characteristic absorption, indicating successful polymer incorporation.

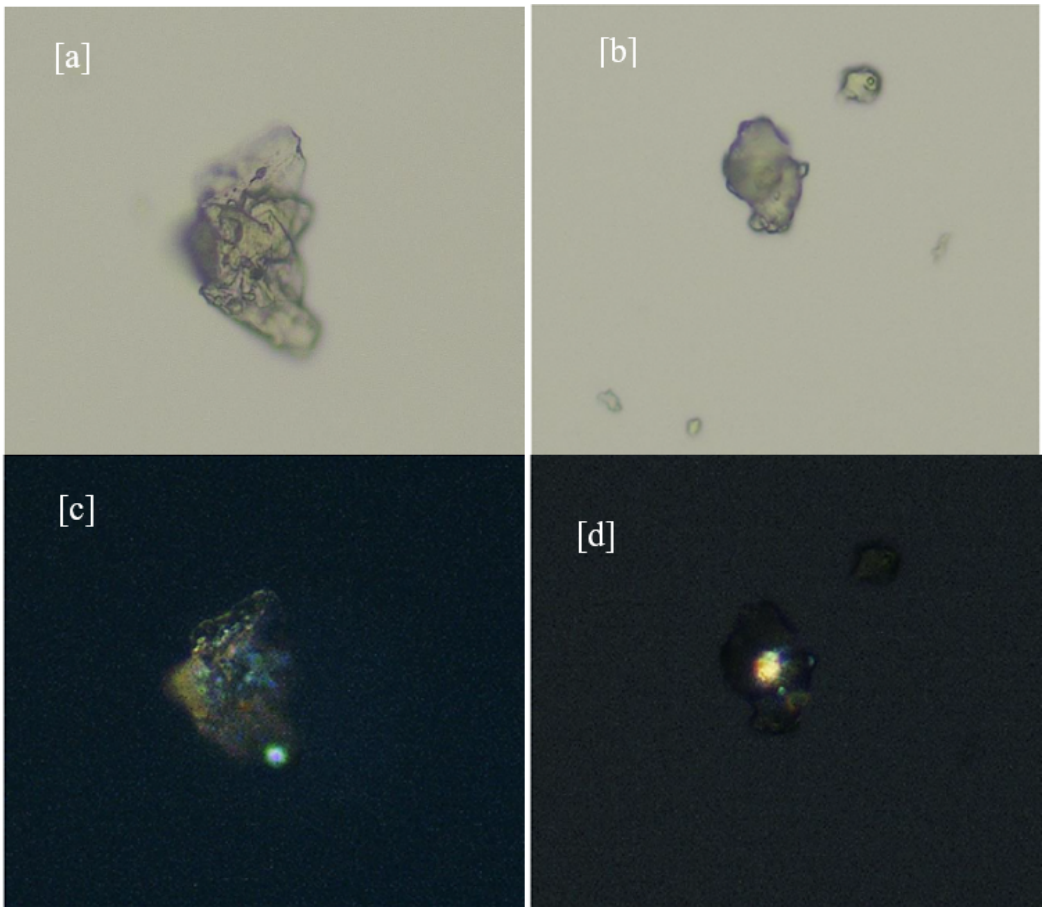

Figure 2. Polarized light microscopy (PLM) images of Smart polymer–AuNPs-UCB-PRP conjugate. (a)(b) show bright field images; (c)(d) show the same fields under crossed polarizers. The presence of birefringent regions (bright areas under polarized light) indicates partial crystallinity or molecular alignment within the composite, likely due to PNIPAM polymer ordering or protein–nanoparticle interactions. Heterogeneous particle morphology and birefringence suggest structural diversity within the conjugate matrix.

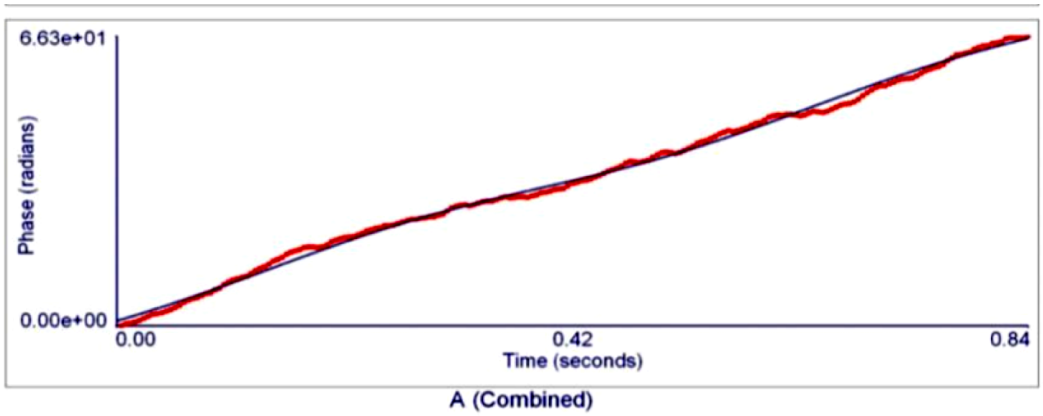

Figure 3. Zeta potential of Smart polymer-AuNPs-UCB-PRP. The red line indicates the measured phase change during electrophoretic mobility assessment, while the black line shows the Smoluchowski model fit.

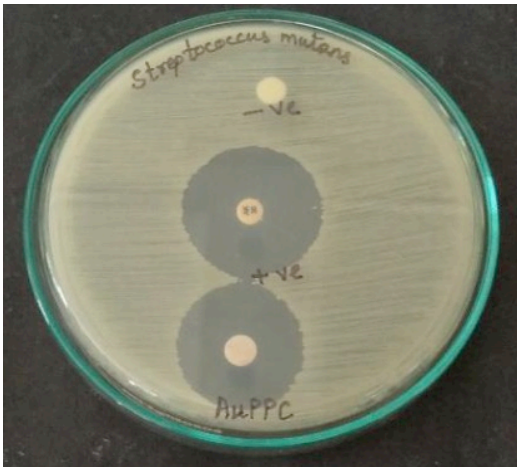

Figure 4. Zone of inhibition showing bacteria free zones around the standard drug and Smart polymer-AuNPs-UCB-PRP. The conjugate exhibited a slightly lower inhibition zone compared to the standard, indicating comparable antimicrobial potential.

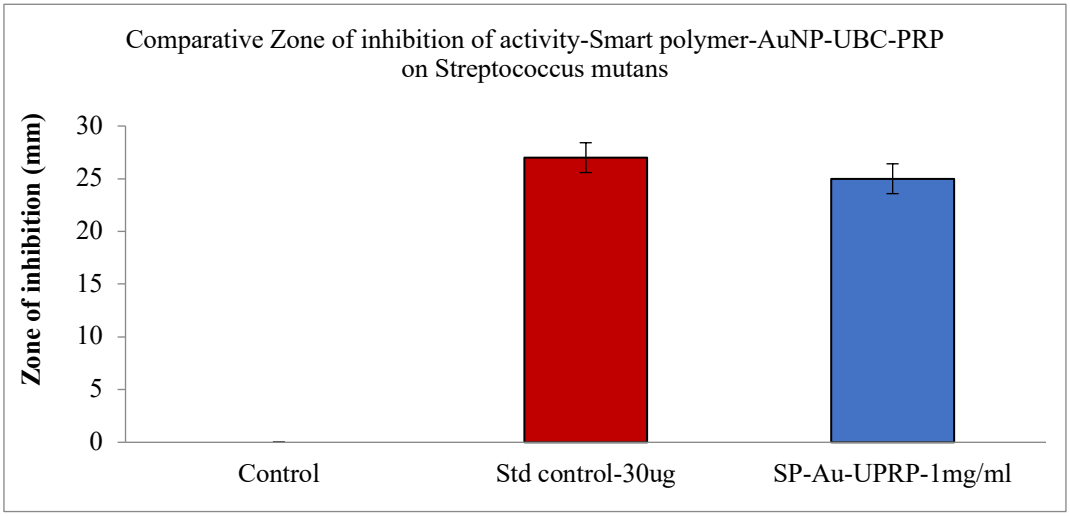

Figure 5. Comparative antibacterial activity of Smart polymer–AuNP–UCB–PRP conjugate against *Streptococcus mutans*. Both treatments demonstrated significant antibacterial activity compared to the untreated control.

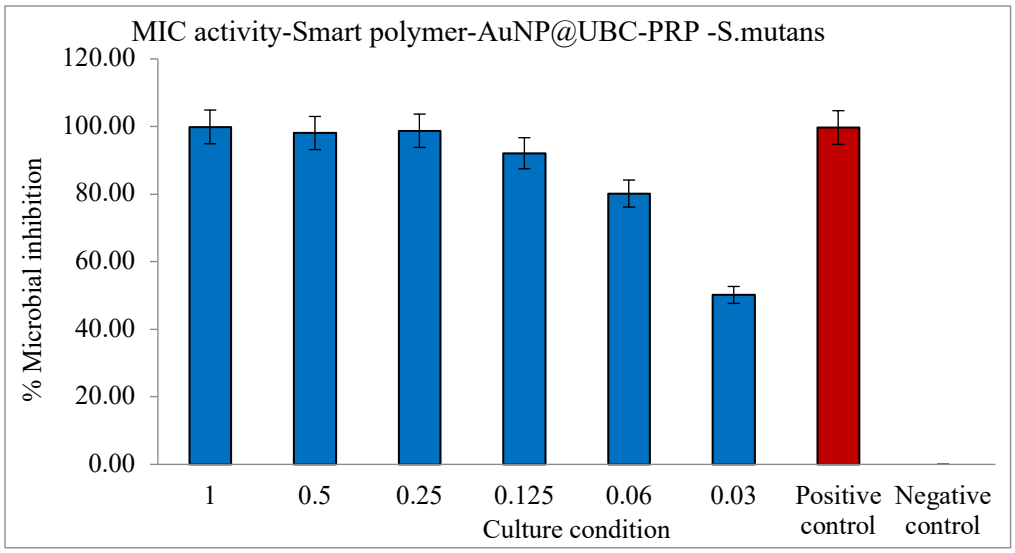

Figure 6. Minimum Inhibitory Concentration (MIC) of Smart polymer–AuNP–UCB–PRP conjugate against *Streptococcus mutans*. The bar graph shows percentage microbial inhibition at different concentrations (1, 0.5, 0.25, 0.125, 0.06, and 0.03 mg/mL) compared with the positive control (standard antibiotic) and negative control (no treatment) indicating concentration-dependent antimicrobial activity.

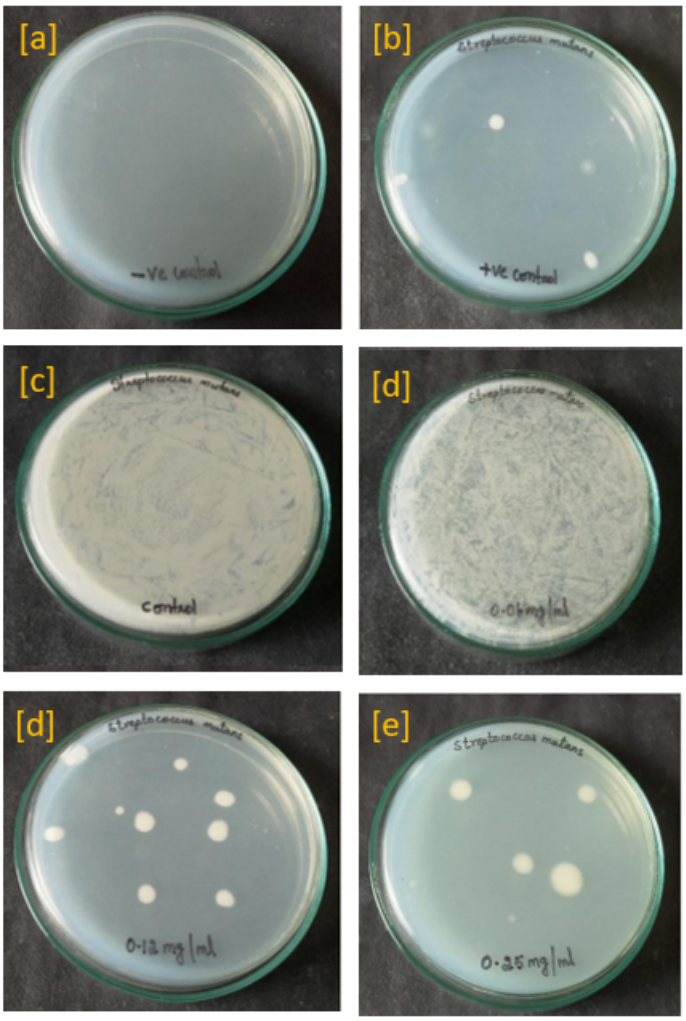

Figure 7. Minimum Bactericidal Concentration assay of Smart polymer-AuNPs-UCB-PRP conjugate against *Streptococcus mutans*. Plates represent: (A) Negative control (no treatment), (B) Positive control (standard antibiotic), (C) Bacterial growth control, (D) 0.06 mg/mL concentration, (E) 0.12 mg/mL concentration, and (F) 0.25 mg/mL concentration. Progressive inhibition zones and reduction in bacterial colonies with increasing concentration indicate dose-dependent bactericidal activity.

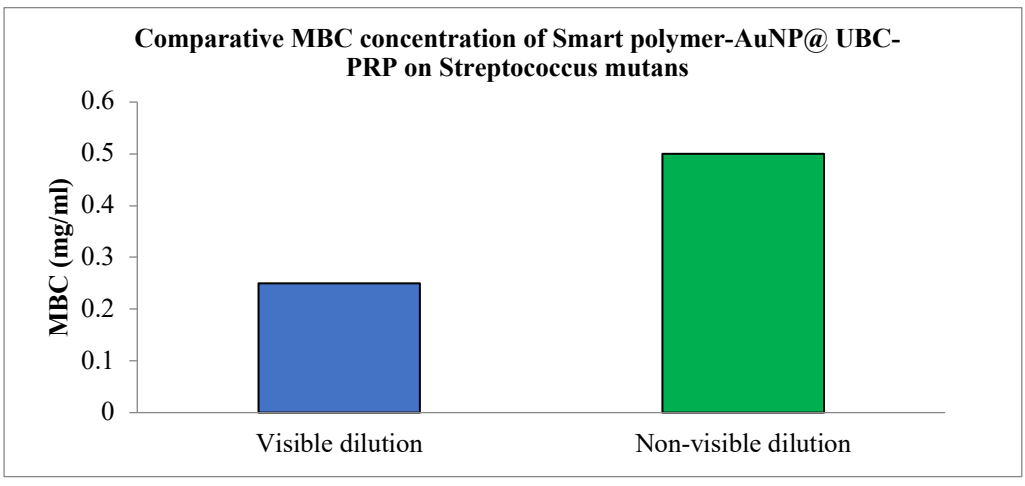

Figure 8. Comparative Minimum Bactericidal Concentration (MBC) of Smart polymer-AuNPs-UCB-PRP against *Streptococcus mutans*. The MBC value was higher for the non-visible dilution (0.5 mg/mL) compared to the visible dilution (0.25 mg/mL), indicating that a greater concentration was required to achieve complete bacterial killing in the absence of visible bacterial growth.

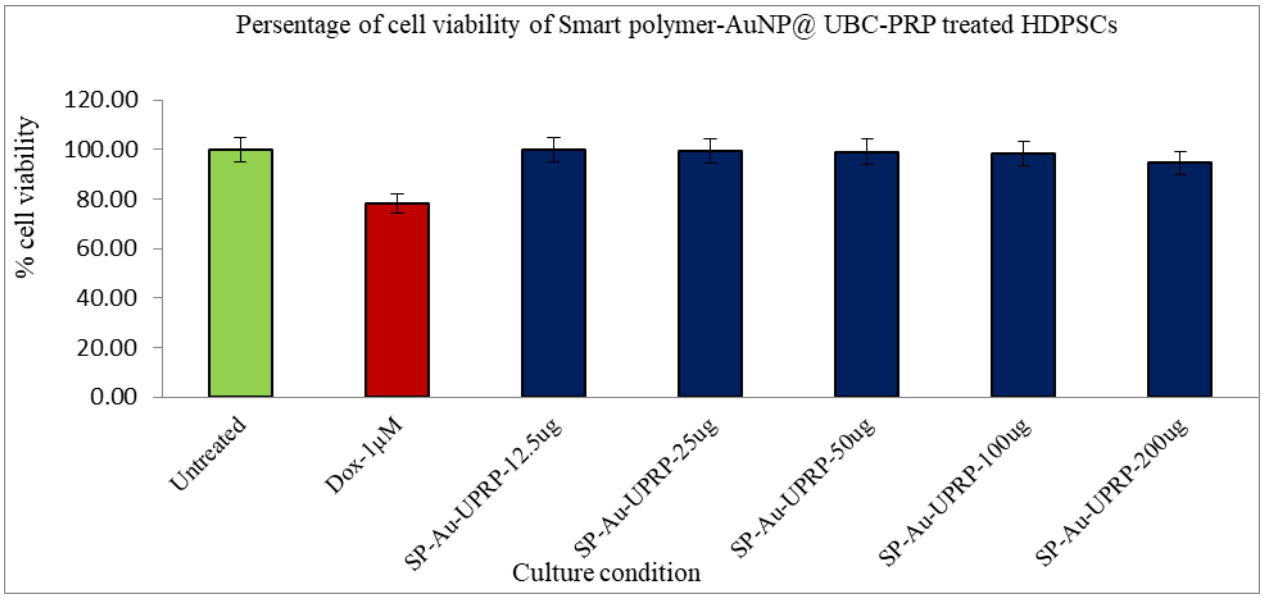

Figure 9. MTT assay showing the percentage cell viability of HDPSCs after treatment with Smart polymer–AuNP@UBC–PRP at varying concentrations (1.25–200 µg). Untreated cells served as the control, while Doxorubicin (1 µM) was used as a positive control for cytotoxicity. Results indicate high cell viability across all tested concentrations of the nanocomposite, suggesting minimal cytotoxicity.

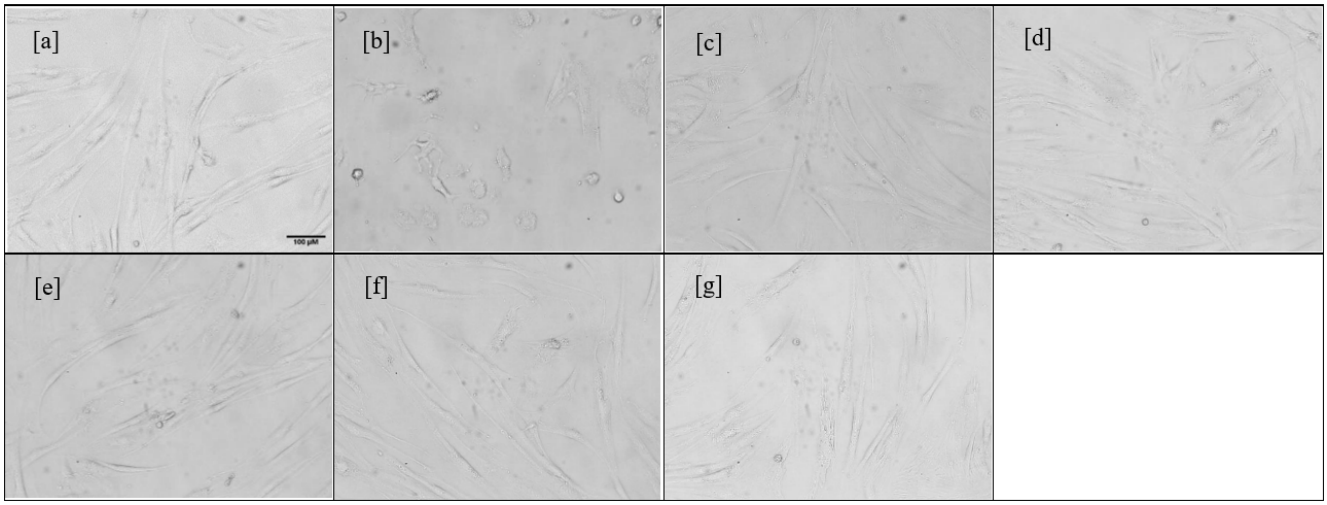

Figure 10: Phase-contrast microscopy images of HDPSCs after 24 h treatment with varying concentrations of Smart polymer-AuNPs-UBC-PRP: (a) Untreated control, (b) Doxorubicin 1 µM (positive control for cytotoxicity), (c) 12.5 µg, (d) 25 µg, (e) 50 µg, (f) 100 µg, and (g) 200 µg. Cells treated with the conjugate maintained normal morphology at all concentrations, indicating minimal cytotoxic effects compared to the positive control.