RESEARCH PAPER

Assessment of Physical Characterization and Cytotoxic Effect of Acidulated Phosphate Fluoride Gel with Gold Nanoparticles on Mesenchymal Stem Cells

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ABSTRACT

Tooth avulsion presents a major clinical challenge and predictably, 2% Acidulated Phosphate Fluoride (APF) has been used to reduce inflammatory root resorption by converting hydroxyapatite to fluoroapatite, though it may cause tissue irritation. Gold nanoparticles (AuNPs) offer a promising alternative due to their osteogenic potential, biocompatibility and minimal toxicity. They can modulate Mesenchymal Stem Cell (MSC) behaviour, exhibit antimicrobial properties and enhance odontoblast activity. AuNPs may help reduce external resorption and promote tissue regeneration. The study aims to evaluate the physical characterization and cytotoxicity of 2% Acidulated Phosphate Fluoride (APF) with AuNPs in-vitro. The methodology was 2% APF-AuNP combination was obtained via centrifugation, followed by characterization using Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) spectroscopy. MSC proliferation was analysed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay over 7, 14, and 21 days, comparing untreated cells, Odontogenic Differentiation Medium (ODM) alone, ODM+APF, and ODM+Au-F. ATR-FTIR showed revealed favourable functional groups suggesting surface modification and increased chemical interactions. Untreated cells retained 100% viability. MTT assay results demonstrated non-cytotoxicity and proliferative effects of APF with AuNP on odontogenic differentiated MSCs. Statistically significant values were observed with ODM+APF values at 21 days when compared to 14 days (P=0.006) and 7 days (P=0.014). ODM+Au-F values at 21 days was statistically significant when compared to 7 days (P=0.036). The MTT assay demonstrated increased MSC viability across all conditions.

Keywords: Acidulated Phosphate Fluoride, Gold nanoparticles, ATR-FTIR, Mesenchymal Stem Cell

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Introduction

Dental avulsion is an injury-induced event characterised by the total displacement of a tooth from its alveolar socket can potentially impacting many tissues, including the periodontal ligament (PDL), alveolar bone, cementum, dental pulp, and gingival mucosa. The most effective action strategy for preserving PDL cell viability in the event of dental avulsion is prompt replantation at the trauma site. It is uncommon to achieve immediate replantation [1]. The duration of extra-alveolar time, the storage media of the tooth and the preservation of the root section all affect PDL cell viability and impact the prognosis for dental replantation [2]. The most serious and frequent side effects following avulsed tooth replantation are inflammatory resorption and replacement resorption following dental alveolar ankylosis [3]. In addition to its antibacterial properties, fluoride acts on the cementum and dentin to change hydroxyapatite into fluoroapatite, which is more resistant to resorption or even prevent the production of clastic cells [4]. This complexity arises from the intricate interplay of various cell types within the periodontium, including MSCs which play a crucial role in tissue repair and regeneration [5].

To effectively harness the regenerative potential of MSCs, researchers have focused on developing novel bioactive materials. These materials serve as scaffolds or delivery vehicles, providing a supportive environment that guides and directs the behaviour of these cells [6]. Bioactive materials play a crucial role in bone tissue engineering by providing a supportive environment for MSCs. They should possess surface properties that encourage cell attachment and proliferation, mimicking the natural extracellular matrix. Moreover, these materials should guide MSC differentiation towards the osteogenic or odontogenic lineage by incorporating biochemical cues that modulate cellular signalling pathways. Finally, they

must provide an environment conducive to osteoblast/odontogenic growth, maturation, and subsequent bone like matrix synthesis and mineralization. Current biomaterials used in bone tissue engineering, such as bioactive glasses, hydroxyapatite, and tricalcium phosphate, often exhibit significant limitations. These materials frequently lack the ability to actively stimulate bone formation, hindering the differentiation of mesenchymal stem cells into bone-forming cells. Furthermore, processing limitations and suboptimal degradation rates can hinder their clinical applicability. To overcome these challenges, the development of novel bioactive materials is crucial [7].

Nanoparticles are increasingly being explored as novel regulators of cell differentiation in the field of regenerative medicine and tissue engineering. These nanoparticles, which include materials such as gold, silver and iron oxide have shown great promise in a variety of biomedical applications. Among them, AuNPs have gained significant attention due to their unique properties and versatility. AuNPs have demonstrated considerable potential in multiple areas of biomedicine, including drug delivery, photothermal and photodynamic therapies, as well as in tissue engineering applications. One of the most notable attributes of gold nanoparticles is their ability to exhibit both antibacterial and antifungal properties, making them highly effective in preventing infection during the healing process. Additionally, AuNPs have been shown to support bone regeneration, a critical aspect in the field of dentistry and orthopaedics. This dual function not only enhances their role in infection control but also promotes tissue repair and regeneration [8].

Recent studies suggest that AuNPs of the correct size and concentration have the ability to influence immune system responses, particularly by modulating macrophage polarization. This modulation can offer significant anti-inflammatory benefits, which is crucial in the context of tissue repair and regeneration. However, there remains a gap in the research regarding how AuNPs interact with immune cells and the subsequent effects on bone regeneration processes. While much of the focus has been on their immunomodulatory properties, less attention has been given to the detailed mechanisms by which these interactions contribute to bone healing and repair, particularly in dental applications such as tooth regeneration and periodontal tissue repair. AuNPs are known for their excellent biocompatibility and minimal toxicity, which makes them particularly advantageous for clinical use. These properties help to promote cellular activity, including odontoblastic differentiation, which is essential for dentin formation and tissue regeneration. Additionally, by enhancing odontoblastic activity, AuNPs can help mitigate the process of resorption, a common issue in dental procedures involving tissue repair. This makes AuNPs a promising material in improving the outcomes of dental treatments, including root resorption management and the promotion of tissue regeneration post-trauma or surgery [9]. In the current study, physical binding and proliferative activity of APF with AuNPs was evaluated.

Methodology

Preparation of 2% APF loaded with gold nanoparticles:

To prepare a homogeneous mixture of AuNPs (15nm) which was obtained from sigma Aldrich (CAS no. 777137) and 2% APF gel, the gold nanoparticle suspension was first centrifuged at 500 rpm for 10 minutes to concentrate the nanoparticles. Subsequently, equal volumes of the concentrated AuNPs suspension and the 2% APF gel were combined in a 1:10 ratio. To obtain a homogenized mixture, to ensure uniform distribution of nanoparticles within the gel matrix, the concentrated AuNPs suspension and the 2% APF gel was micro pipetted in an eppendorf tube and the mixture was subjected to centrifugation at 1000 rpm for 1 minute which ensures a consistent and homogeneous preparation of 2% APF gel with AuNPs. The final mixture was stored at 4^{o}C until further use.

Characterization using ATR-FTIR spectrometry technique:

In this study 2% APF gel with AuNPs, ATR- FTIR can provide insights into the chemical interactions between these materials. A small volume of the gold nanoparticle solution and the gold nanoparticle-infused 2% APF gel was deposited onto the surface of the ATR crystal. The ATR crystal with the sample was then positioned on the FTIR instrument. The infrared spectrum was scanned within the desired wavelength range, focusing on the mid-infrared (fingerprint) region to identify functional groups. The absorbance spectrum was recorded, representing the intensity of the infrared radiation absorbed by the molecules at different wavelengths.

Proliferation of odontoblastic cells using MTT Assay:

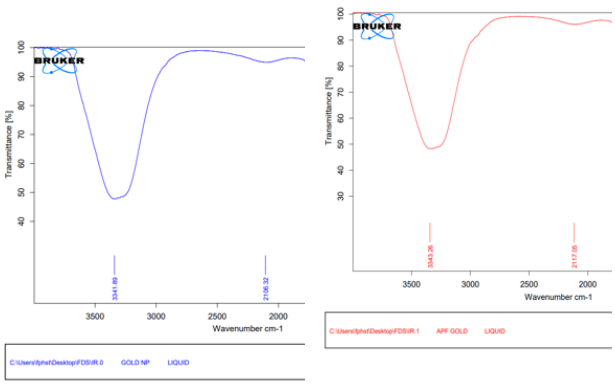
The MTT assay was performed using HiFi Mesenchymal Stem Cells (HiFi MSCs) procured from HiMedia Labs, Mumbai, India. The cells were maintained in HiMesoXL Mesenchymal Stem Cell Expansion Medium supplemented with 10% FBS and 1% antibiotic-antimycotic solution. Cultures were kept at 37°C in a humidified atmosphere of 5% CO_2 and 18–20% O_2, and sub-cultured every two days. Passage 4 cells were used for the experiment. For the assay, 1000 µL of cell suspension containing 5,000 cells per well was seeded into a 12-well plate and allowed to grow for 48 hours. After removing the spent medium, the cells were stimulated with Odontogenic Differentiation Medium (ODM), containing 100 nM/L

Dexamethasone, 50 mg/mL L-Ascorbic acid, and 10 mM/L beta-glycerophosphate in HiMesoXL medium, along with 50 µL of undiluted experimental compounds (APF and AUF). The cells were incubated for 7, 14 and 21days with the medium replaced every 2–3 days. After the incubation period, the spent medium was discarded and 500 µL of MTT reagent (0.5 mg/mL) was added to each well and incubated at 37°C for 3 hours. The MTT reagent was then removed, and 500 µL of DMSO was added to dissolve the formazan crystals. Absorbance was measured at 570 nm using a microplate reader. Controls included medium-only wells (medium control), wells with cells but no experimental compound (negative control), and wells with cells treated with ODM alone (positive control). The assay was conducted in triplicate.

Cell viability was calculated using the formula:

% cell viability = [Mean abs of treated cells/Mean abs of Untreated cells] x 100.

Results



(a) (b)

Fig. 1 (a)ATR FTIR of Gold, (b) ATR FTIR of APF Gel and Gold.

Table. 1 Comparison of ODM alone, ODM + APF, ODM+AUF between 7th day, 14th day and 21st day

		Mean	SD	F	P value	η2 value
ODM alone	7 days	100.45	0.35	1342.72	0.001*	1
	14 days	105.29	0.4			
	21 days	107.68	0.65			
ODM+APF	7 days	101.35	0.27	174.36	0.001*	0.99
	14 days	106.88	1.35			
	21 days	112.49	0.71			
ODM+AU-F	7 days	101.46	1.67	49.08	0.002*	0.96
	14 days	103.87	1.36			
	21 days	111.1	0.42			

*Statistical significance set at 0.05; **N:** Number of samples; **SD:** Standard deviation; ***η*2 value:** Eta value

Table 2: Multiple **c**omparison of groups between 7th day, 14th day and 21st day using Bonferroni post-hoc analysis.

		Mean diff.	Std. Error	t	p	95% CI lower limit	95% CI upper limit
7 days ODM alone	14 days ODM alone	-4.84	0.064	-76.053	.001*	-5.11	-4.56
7 days ODM alone	21 days ODM alone	-7.23	0.182	-39.691	.002*	-8.02	-6.45
14 days ODM alone	21 days ODM alone	-2.4	0.153	-15.664	.012*	-3.06	-1.74
7 days ODM+ APF	14 days ODM+ APF	-5.54	0.826	-6.707	.065	-9.09	-1.98
7 days ODM+ APF	21 days ODM+ APF	-11.14	0.487	-22.883	.006*	-13.24	-9.05
14 days ODM+ APF	21 days ODM+ APF	-5.61	0.387	-14.494	.014*	-7.27	-3.94
7 days ODM+ AU-F	14 days ODM+ AU-F	-2.41	0.938	-2.572	.371	-6.45	1.62

7 days ODM+ AU-F	21 days ODM+ AU-F	-9.64	1.067	-9.036	.036*	-14.24	-5.05
14 days ODM+ AU-F	21 days ODM+ AU-F	-7.23	1.029	-7.027	.059	-11.66	-2.8

*Statistical significance set at 0.05. Bonferroni post-hoc analysis displayed higher ODM alone values at 21 days when compared to 14 days (Mean difference = -7.23; P=0.001) and 7 days (Mean difference = -2.4; P=0.012). Similarly, higher ODM alone values at 14 days when compared to 7 days (Mean difference = -4.84; P=0.001). Higher ODM+APF values at 21 days when compared to 14 days (Mean difference = -11.14; P=0.006) and 7 days (Mean difference = -5.61; P=0.014). Analysis displayed higher ODM+ Au-F values at 21 days when compared to 7 days (Mean difference = -9.64; P=0.036).

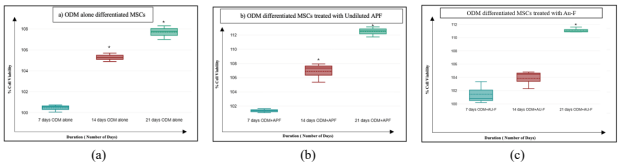


Fig. 2. Graphs represented the % cell viability values of a) ODM alone differentiated MSCs b) ODM differentiated MSCs treated with Undiluted APF and c) ODM differentiated MSCs treated with Au-F after the incubation period of 7, 14 and 21days treatment along with untreated cells.

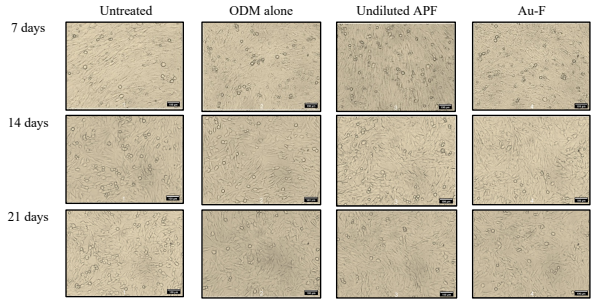


Fig.3: Overlaid montage photo represented the morphology of untreated MSCs, ODM alone differentiated MSCs, ODM differentiated MSCs treated with Undiluted APF, ODM differentiated MSCs treated with Au-F after the incubation period of 7,14 and 21days of incubation. All the images were captured under inverted biological microscope at 20x magnification and recorded with the help of MICAM software. Scale bar: 200µm.

Discussion

The study employed ATR-FTIR spectroscopy to analyze the interactions between AuNP and a 2% APF solution. A peak around 2900 cm^{-1} was identified, corresponding to C-H stretching typical of alkanes or organic compounds. Additionally, a strong peak near 1700 cm^{-1} suggested C=O stretching, indicative of carbonyl groups such as ketones, aldehydes or carboxylic acids. The fingerprint region between 1500 and 1000 cm^{-1} contained various peaks that may correspond to C-O, C-C, or other complex vibrations. The ATR-FTIR analysis revealed characteristic peaks associated with functional groups present in both the APF gel and the gold nanoparticles. The peaks around 3300 cm^{-1} indicated O-H stretching, suggesting the presence of hydroxyl groups that could facilitate adhesion to tooth enamel. The presence of C=O stretching peaks near 1700 cm^{-1} indicated carbonyl-containing compounds, which may have played a role in binding interactions with the tooth surface. Moreover, the fingerprint region (1000–1500 cm^{-1}) provided detailed information about the chemical composition and structural changes that occurred upon interaction with tooth enamel. Changes in peak intensity or shifts in wavenumbers indicated that gold nanoparticles enhanced the binding capacity of APF by modifying the surface properties of the enamel or by promoting chemical interactions.

The results revealed two distinct Fourier-transform infrared (FTIR) spectra, each representing the infrared absorption characteristics of the samples across a wavenumber range of 4000–400 cm^{-1}.In the first spectrum, attributed to pure gold (Fig.1(a)), notable peaks were observed at approximately 3300 cm^{-1}, indicating a broad peak for O-H stretching, likely due to hydroxyl groups or water. The second spectrum (Fig.1(b)), representing the APF gel with gold nanoparticles, displayed similar peaks at around 3300 cm^{-1} for O-H stretching and 2900 cm^{-1} for C-H stretching vibrations. However, the C=O stretching peak at approximately 1700 cm^{-1} appeared sharper compared to that in the gold spectrum. The fingerprint region in this spectrum was more defined than in the first, suggesting structural or compositional differences due to the incorporation of gold nanoparticles into the APF gel.

The MTT cell viability assay demonstrated APF gel and Au-F, exhibited both non-cytotoxic and cell-proliferative properties when applied to odontogenic differentiated MSCs. Cells treated with these compounds consistently showed higher viability values compared to untreated controls throughout the incubation periods. This increase in cell viability underscores the ability of the compound to support cellular proliferation confirming their beneficial effects on ODM-induced MSCs over time.

The undiluted samples of APF and Au-F demonstrated the most significant proliferative effects, maintaining optimal cell viability throughout the study without any observable decline. This consistency highlights the stability and

efficacy of these compounds in promoting cell proliferation under the experimental conditions. The results were statistically significant at the conventional alpha levels of 0.05, indicating that there are significant differences among the means of 7, 14 and 21 days for ODM alone and ODM+APF of the treatment. Correspondingly, for ODM+APF the p-value of <.001, the results are statistically significant at the conventional alpha levels of 0.05, with significant differences among the means 7 ,14 and 21 days of incubation (Table. 1).

The results showed that MSCs treated with medium (control) maintained high viability, while cells treated with ODM alone exhibited slightly high viability compared to the control group across the time period. Bonferroni post-hoc analysis displayed ODM alone statistically significant values at 21 days when compared to 14 days (Mean difference = -7.23; P=0.001) and 7 days (Mean difference = -2.4; P=0.012). Similarly, higher ODM alone values at 14 days when compared to 7 days which was also statistically significant (Mean difference = -4.84; P=0.001) (Table.2). Higher ODM with APF values at 21 days when compared to 14 days (Mean difference = -11.14; P=0.006) and 7 days (Mean difference = -5.61; P=0.014). ODM with Au-F values were higher at 21 days when compared to 7 days was statistically significant (Mean difference = -9.64; P=0.036). MSCs exposed to ODM followed by treatment with Au-F displayed a notable significant cell viability at 21 days.

Morphological observations of MSCs under an inverted biological microscope at 20x magnification revealed significant differences between the non-treated control and treated groups. Untreated MSCs maintained their typical fibroblast-like spindle morphology, while cells subjected to ODM followed by APF or Au-F treatments showed no morphological changes (Fig.3). These findings suggest that undiluted APF and Au-F treatments have a noticeable impact on the viability and morphology of ODM-differentiated MSCs after incubation period.

The International Dental Traumatology Association has recommended that avulsed teeth with fully developed roots that are replanted after an extraoral period longer than 60 minutes be submerged in 2.4% APSF (pH 5.5) for five minutes following extensive removal of PDL remnants [10].Bone Marrow-derived Mesenchymal Stem Cells (BM-MSCs) has been demonstrated to be stimulated by AuNPs in combination with various delivery strategies [11, 12]. The ability of DPSCs to develop into osteogenic lineage [13] or as nanocomposite 3D culture [14,15] was evaluated by treatments with AuNPs alone. Similarly, dental stem cells generated from periodontal ligaments (PDLSCs) underwent size-dependent osteogenic differentiation triggered by AuNPs [16,17,18]. Regardless of size, AuNPs either alone or in combination with other biomaterials encouraged MSC development toward the osteogenic lineage in Adipose-derived Stem Cells (ASCs)[19,20,21,22].

The MTT cell viability results suggest us that APF and Au-F were non-cytotoxic as well as cell proliferative in nature on odontogenic differentiated MSCs with increased cell viability values than the untreated cells and confirmed the cell proliferative effect till the 7days, 14days and 21days. Undiluted samples maintained best proliferative effect on ODM induced MSCs without losing rate of viability values till 7days, 14days and 21days increased exponentially concluding that it has better odontoblastic effects on ODM induced MSCs.

Conclusion

The study investigates the potential of gold nanoparticles (AuNPs)when added to fluoride gel has promising results on ODM induced MSCs in-vitro. Gold nanoparticles significantly improve the binding capacity of Acidulated Phosphate Fluoride (APF) by modifying the surface properties of tooth enamel or promoting chemical interactions. ATR-FTIR spectroscopy revealed notable spectral changes indicating enhanced adhesion characteristics between the APF and AuNPs. The APF gel and APF with AuNP (Au-F) were found to be non-cytotoxic to odontogenic differentiated MSCs. The MTT assay demonstrated that APF gel and Au-F promoted cell proliferation, with increased cell viability observed at 14 and 21 days compared to untreated cells. The study concluded that Au-F showed statistically significant difference in enhancing the odontogenic differentiation of MSCs at 21days suggesting their possible applicability of surface treatment of avulsed tooth in delayed implantation cases.

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