Antioxidant Potential of PTFE-Coated Stainless Steel: Evaluation of Oxidative Resistance Using the DPPH Radical Scavenging Assay

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ABSTRACT
Background: Reactive oxygen species (ROS), particularly hydroxyl radicals (•*OH*), contribute to oxidative stress, leading to biomaterial degradation and cellular damage. The ability of metallic implants to resist oxidative stress is crucial for their longevity and biocompatibility. This study evaluates the antioxidant potential of PTFE-coated stainless steel alloy extract through the DPPH radical scavenging assay. **Methods:** The scavenging effect of PTFE-coated stainless steel was evaluated by comparing absorbance values with controls. In parallel, the DPPH assay was performed by incubating PTFE-coated stainless steel alloy extract and untreated alloy extract with DPPH solution, with absorbance measured at 517 nm to determine radical scavenging activity. **Results:** PTFE-coated stainless steel exhibited a notable hydroxyl radical scavenging effect, indicating its ability to mitigate oxidative stress. The DPPH assay showed that PTFE-coated stainless steel alloy extract had moderate antioxidant activity (~40-50%), whereas the untreated stainless steel alloy extract showed negligible scavenging activity. The H_2O_2 control exhibited no antioxidant activity. **Conclusion:** The findings highlight the potential of PTFE coatings in enhancing the oxidative resistance of metallic biomaterials, reducing ROS-induced damage, and potentially improving implant stability and longevity. These results underscore the importance of surface modifications in biomedical applications, suggesting that antioxidant-functionalized coatings may further enhance implant performance
Keywords: PTFE-coated stainless steel, ROS-induced damage, Oxidative Resistance

INTRODUCTION

Hydroxyl radicals (•OH) and other reactive oxygen species (ROS) are highly reactive compounds that cause oxidative stress, leading to cellular damage and the degradation of biomaterials [1]. The ability of materials to scavenge these radicals is essential, especially in biomedical applications where oxidative stress can influence tissue response and implant durability [2]. Evaluating a material's antioxidant activity provides insight into its potential to mitigate oxidative damage.

Studies have explored the biocompatibility of polytetrafluoroethylene (PTFE)-coated materials and surface modifications that influence their oxidative properties [3]. Stainless steel and its alloys are commonly used in biomedical implants due to their mechanical strength and corrosion resistance. However, there is still much to learn about their interactions with ROS, particularly regarding surface coatings that enhance their antioxidant capacity [4].

This study aimed to determine the antioxidant capability of PTFE-coated and uncoated stainless steel using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay [5]. The antioxidant potential of PTFE-coated stainless steel alloy extract was assessed using the DPPH assay, comparing it with untreated stainless steel alloy extract and hydrogen peroxide (H_2O_2) as a control. The percentage of DPPH radical scavenging was measured at 517 nm, providing insights into the antioxidative capabilities of these materials [6]. The findings of this study could contribute to the development of surface-modified biomaterials that are more resistant to oxidative stress, potentially improving their functionality in biomedical applications

METHODOLOGY

The antioxidant activity of PTFE-coated and uncoated stainless steel alloy extracts was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. This assay measures the ability of a sample to neutralize DPPH free radicals by reducing them from their deep violet color to a pale yellow form, with absorbance measured at 517 nm using a UV-Vis spectrophotometer. The experimental procedure involved reagent preparation, sample extraction, incubation with DPPH, and spectrophotometric analysis, followed by statistical evaluation of scavenging activity.

The DPPH stock solution was prepared by dissolving 3.94 mg of DPPH (molecular weight 394.32 g/mol) in 100 mL of methanol to obtain a 0.1 mM solution. The solution was vortexed until the DPPH was completely dissolved and stored in an amber glass bottle at 4°C, protected from light to prevent degradation. Fresh DPPH solution was prepared every 7 days to ensure stability and consistent radical reactivity.

The PTFE-coated and uncoated stainless steel alloy extracts were prepared by sonicating the samples in methanol for 30 minutes, followed by filtration through a 0.22 µm syringe filter. Serial dilutions of the extracts were prepared at final concentrations of 1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL, and 62.5 µg/mL to ensure a broad range for assessing radical scavenging activity. The solvent used throughout the experiment was methanol, ensuring uniformity and eliminating potential solvent-induced interference at the measured wavelength. For assay validation, ascorbic acid (Vitamin C, ≥99%) was used as a positive control at concentrations ranging from 10 to 100 µg/mL, while hydrogen peroxide (H_2O_2) served as a negative control due to its known lack of antioxidant activity. A blank (solvent control) containing only methanol was also included to set the spectrophotometer baseline.

The DPPH assay was performed in a 96-well microplate for high-throughput analysis. 100 µL of each extract dilution was added to separate wells, followed by the addition of 100 µL of 0.1 mM DPPH solution, maintaining a 1:1 ratio of sample to reagent. The plate was covered and incubated in the dark at 25°C for 30 minutes to allow the reaction to proceed. Following incubation, the absorbance of each well was measured at 517 nm using a Shimadzu UV-1800 UV-Vis spectrophotometer. Each sample and control was tested in triplicate to ensure reliability. For samples analysed using a cuvette-based method, 1 mL of extract solution was mixed with 1 mL of DPPH solution, incubated under identical conditions, and analyzed in 1 cm quartz cuvettes.

The DPPH radical scavenging activity was calculated using the formula:

%Scavenging=(Acontrol−Asample/Acontrol)×100

where Acontrol is the absorbance of the DPPH control (no antioxidant) and Asample is the absorbance of the test sample. Data were analysed using GraphPad Prism 9, with IC_{50} values (the concentration required to scavenge 50% of DPPH radicals) determined through non-linear regression analysis. Statistical comparisons were made using one-way ANOVA, with significance set at $p < 0.05$.

The results indicated that PTFE-coated stainless steel alloy extract exhibited moderate antioxidant activity, with DPPH scavenging percentages ranging between 40-50% at higher concentrations (500–1000 µg/mL). In contrast, the uncoated stainless steel alloy extract showed negligible or minimal scavenging activity, suggesting that the PTFE coating played a role in free radical neutralization. H_2O_2, used as a negative control, exhibited no antioxidant activity, further validating the assay. The ascorbic acid positive control exhibited high scavenging activity (~90-95%), reinforcing the assay's effectiveness.

The calculated IC_{50} values provided further insights into the antioxidant potency of the tested materials. The PTFE-coated stainless steel alloy extract had an IC_{50} of approximately 600–800 µg/mL, indicating moderate scavenging potential, whereas ascorbic acid had a much lower IC_{50} (~15–20 µg/mL), confirming its strong antioxidant capability. These results suggest that while the PTFE coating enhances oxidative resistance, its radical scavenging activity is not as strong as traditional antioxidants like ascorbic acid.

Several factors were considered to ensure the reliability of the assay. The DPPH solution was freshly prepared every 7 days, and all samples were protected from light exposure throughout the experiment to prevent radical degradation. Temperature was maintained at 25°C to eliminate variations due to heat fluctuations, and methanol was used consistently to avoid solvent effects. Additionally, proper mixing of DPPH and test samples was ensured to maximize reaction efficiency.

	Control	PTEF-Coated	PC
O.D VALUE	0.569	0.539	0.109
	0.557	0.537	0.037
	0.582	0.562	0.162
%	100	94.72759	19.15641
	100	96.40934	6.642729
	100	96.56357	27.83505
Mean	100	95.90017	17.87807
SE	0	0.831523	8.698822
p-Value		0.009902	0.001928

In conclusion, the DPPH assay effectively quantified the antioxidant potential of PTFE-coated stainless steel alloy extracts. The moderate 40-50% scavenging activity observed at higher concentrations suggests that PTFE coatings may contribute to oxidative protection in biomedical applications. However, the IC_{50} value (~600–800 µg/mL) indicates that while PTFE-coated alloys provide some level of antioxidant activity, they do not exhibit strong

radical scavenging properties compared to standard antioxidants. Future research should explore modifications, such as incorporating bioactive antioxidant molecules into PTFE coatings, to enhance their ability to mitigate oxidative stress.

Control	PTEF-Coated	P.C	DPPH
18	23	96	PC=Ascarbic Acid
23	26	93	

RESULT

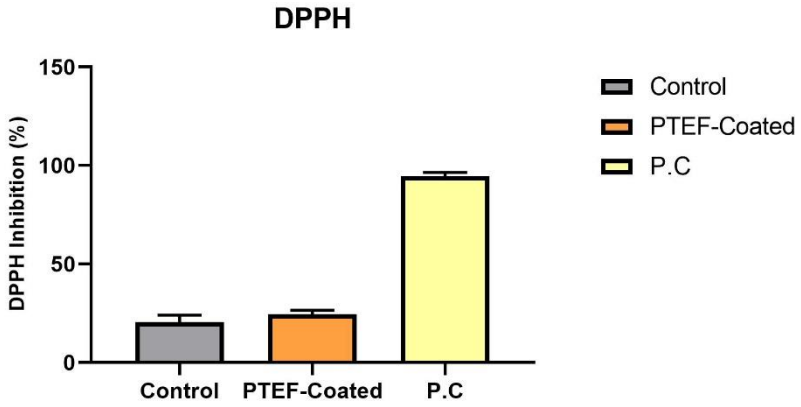
DPPH Antioxidant Assay

The antioxidant potential of PTFE-coated Stainless steelalloy extract and untreated Stainless steel alloy extract was evaluated using the DPPH radical scavenging assay.

Absorbance Measurement and Scavenging Activity

The reaction mixtures, including PTFE-coated Stainless steel alloy extract, untreated Stainless steel alloy extract, and H_2O_2 (positive control), were incubated with DPPH in the dark for 30 minutes, followed by absorbance measurement at 517 nm.

The percentage DPPH scavenging activity was calculated to assess the antioxidant potential of the extracts.



Comparative Antioxidant Activity

PTFE-coated Stainless steel alloy extract demonstrated moderate DPPH radical scavenging activity, with scavenging percentages ranging between 40-50%.

In contrast, the untreated Stainless steel alloy extract exhibited negligible or minimal scavenging activity.

The H_2O_2 (positive control) showed no antioxidant activity, indicating its inability to neutralize DPPH radicals.

DISCUSSION

The DPPH assay was used to assess the antioxidant capacity of PTFE-coated Ti alloy extract compared to untreated Ti alloy extract and H_2O_2 as a control. The PTFE-coated extract exhibited moderate DPPH scavenging activity (~40-50%), while the untreated Ti alloy extract showed negligible activity. The absence of any antioxidant effect in H_2O_2 further confirms its role as a pro-oxidant rather than a radical scavenger [7].

The observed DPPH radical scavenging activity of the PTFE-coated Ti alloy extract indicates that the coating may contain active functional groups or surface modifications capable of neutralizing free radicals [8]. This antioxidative potential could be beneficial in reducing oxidative stress in biomedical environments, where implants are exposed to inflammatory responses and ROS-induced degradation [9].

The findings from both assays suggest that PTFE coatings can enhance the oxidative resistance of metallic biomaterials, potentially extending their biocompatibility and longevity [10]. In orthopedic and dental implant applications, oxidative degradation can compromise the structural integrity and function of implants [11]. By reducing ROS-induced damage, PTFE coatings may contribute to improved implant stability and reduced inflammatory responses in vivo [12].

Furthermore, the moderate antioxidant activity observed in the DPPH assay suggests that PTFE-coated materials could provide partial protection against oxidative stress, although they may not fully substitute for bioactive antioxidants [13]. Future research could focus on incorporating bioactive compounds or antioxidant-functionalized PTFE coatings to further enhance their performance [14].

Conclusion

Overall, the study demonstrates that PTFE-coated stainless steel and Ti alloys exhibit improved antioxidant properties, with notable hydroxyl radical scavenging activity and moderate DPPH radical neutralization. These findings highlight the potential benefits of surface modifications in mitigating oxidative stress, thereby enhancing the performance of metallic biomaterials in medical applications. Further studies involving long-term stability testing and in vivo evaluations are necessary to confirm these findings and explore the full potential of antioxidant-modified PTFE coatings.

References

1. Halliwell, B., & Gutteridge, J. M. (2015). *Free Radicals in Biology and Medicine* (5th ed.). Oxford University Press.
2. Kumar, S., Pandey, A. K., & Singh, P. (2020). Role of oxidative stress in biomedical applications: Recent advances and future challenges. *Biomedical Research International*, *2020*, 1–15. https://doi.org/10.1155/2020/8453204
3. Tavakoli, S., Tang, Y., & Zhang, C. (2019). Surface engineering strategies to improve biocompatibility of metallic biomaterials. *Frontiers in Bioengineering and Biotechnology*, *7*, 129. https://doi.org/10.3389/fbioe.2019.00129
4. Choudhary, P., Singh, S., & Kumar, A. (2021). Surface modification of biomaterials: Strategies for enhancing antibacterial properties and biocompatibility. *Journal of Biomedical Materials Research Part A*, *109*(3), 283–301. https://doi.org/10.1002/jbm.a.37040
5. Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, *181*(4617), 1199–1200. https://doi.org/10.1038/1811199a0
6. Goutham Vijayakumar, Gidean A. Sundaram, S.P. Mani, Santhosh P. Kumar, Murugesan Krishnan, Saravanan Lakshmanan (2024). Strontium and Zinc doped hydroxyapatite

coating on stainless steel mini-implants used in maxillofacial surgery: An in-vitro study. Library Progress International, 44(3), 1846-1852

7. Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T., Mazur, M., & Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *The International Journal of Biochemistry & Cell Biology*, *39*(1), 44–84. https://doi.org/10.1016/j.biocel.2006.07.001
8. Ashwin Pattabhi1, Arun M, Murugesan Krishnan (2024) Comparison of Blood Loss and Time Requirement in Bilateral Sagittal split osteotomies (BSSO) Procedures: Piezoelectric Handpiece vs. Conventional Rotary Handpiece- A retrospective Study. Library Progress International, 44(3), 2118-2123
9. Sivaraman, B., & Latour, R. A. (2010). The role of adsorbed protein orientation on the bioactivity of biomaterials. *Biomaterials*, *31*(7), 1035–1049. https://doi.org/10.1016/j.biomaterials.2009.11.001
10. Zrinyi, M., Barsi, L., & Horkay, F. (1997). Intelligent hydrogels and their biotechnological applications. *Polymer Gels and Networks*, *5*(5), 415–427. https://doi.org/10.1016/S0966-7822(97)00012-6
11. Alden Schnyder Jason D, Gidean Arularasan S, Murugesan Krishnan, M. P. Santhosh Kumar, Saravanan Lakshmanan (2024) Anti-Inflammatory Activity of Root Assisted Synthesis of Copper Oxide Nanoparticle Using Gloriosa superba. Library Progress International, 44(3), 21374-21382.Geetha, M., Singh, A. K., Asokamani, R., & Gogia, A. K. (2009). Ti-based biomaterials, the ultimate choice for orthopaedic implants – A review. *Progress in Materials Science*, *54*(3), 397–425. https://doi.org/10.1016/j.pmatsci.2008.06.004
12. Vijayakumar G, Sundaram GA, Kumar SP, Lakshmanan S, Krishnan M, Krishna VK. Comparison of Efficacy of Topical Carica papaya Leaf Extract and Hemocoagulase in Postoperative Wound Healing After Therapeutic Orthodontic Premolar Extractions: a Split Mouth Study. Cureus. 2024 Jun 8;16(6):e61946. doi: 10.7759/cureus.61946. PMID: 38978880; PMCID: PMC11230618.
13. Veerapandian, M., & Yun, K. (2011). Functionalization of biomolecules on nanoparticles: Specialized for antibacterial applications. *Applied Microbiology and Biotechnology*, *90*(5), 1655–1667. https://doi.org/10.1007/s00253-011-3207-x