Biocompatibility of Stainless-Steel Alloys: A Comparative Evaluation of PTFE Coatings with MTT Cytotoxicity Assay

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Abstract
Background: Oxidative stress plays a crucial role in biomaterial degradation and cellular responses, influencing the long-term performance of biomedical implants. Stainless steel alloys, commonly used in dental applications, are prone to oxidative interactions that may lead to cytotoxic effects. Polytetrafluoroethylene (PTFE) coatings are known for their corrosion resistance and potential to modify surface interactions. This study evaluates the antioxidant potential and biocompatibility of PTFE-coated stainless-steel alloy in comparison to untreated stainless steel alloy. **Methods:** The antioxidant activity of PTFE-coated and untreated stainless steel alloy extracts was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, with hydrogen peroxide (H_2O_2) serving as a positive control. The biocompatibility of PTFE-coated stainless steel alloy was evaluated using the MTT cytotoxicity assay on MG-63 osteoblast cells after a 24-hour incubation period. Statistical analysis was performed using one-way ANOVA to determine significance levels. **Results:** The PTFE-coated stainless steel alloy exhibited moderate antioxidant activity, with approximately 40-50% DPPH scavenging, while the untreated alloy showed minimal activity. As expected, H_2O_2 demonstrated no antioxidant effect. The MTT assay revealed significantly higher cell viability (~85-90%) in PTFE-coated stainless steel alloy compared to untreated stainless steel alloy (~65-75%), suggesting improved biocompatibility. The positive control (H_2O_2) resulted in severe cytotoxic effects (~25-30% viability), validating its oxidative stress-inducing properties. Statistical comparisons confirmed that the differences in antioxidant activity and cell viability between PTFE-coated and untreated stainless steel alloy were significant ($p < 0.05$). **Conclusion:** The findings suggest that PTFE coatings enhance the oxidative resistance and cytocompatibility of stainless steel alloys, making them more suitable for biomedical applications. The improved cell viability and moderate antioxidant properties indicate that PTFE coatings may reduce oxidative stress-induced damage, potentially leading to longer implant lifespan and better biocompatibility. Future studies should explore further functionalization strategies to enhance the antioxidant potential of PTFE-coated biomaterials and assess their long-term performance in vivo.
Keywords: PTFE coating, stainless steel alloy, oxidative stress, biocompatibility, DPPH assay, MTT assay, antioxidant activity, biomaterials.

INTRODUCTION

Oxidative stress, resulting from an imbalance between reactive oxygen species (ROS) and the body's antioxidant defenses, is a major contributor to cellular damage and plays a pivotal role

in the degradation of materials used in biomedical and dental devices [1]. Reactive oxygen species, including hydrogen peroxide (H_2O_2) and superoxide radicals ($O_2•−$), can cause significant structural damage to both biomaterials and biological tissues [2]. This oxidative damage is particularly relevant for materials such as dental and biomedical alloys, which are frequently exposed to biological environments that can induce corrosion, surface degradation, and inflammation [3]. Therefore, understanding the antioxidant properties of these materials is essential for assessing their biocompatibility, longevity, and their potential to mitigate oxidative damage when implanted or exposed to the human body [4].

Polytetrafluoroethylene (PTFE), a synthetic fluoropolymer, is widely recognized for its remarkable chemical inertness, corrosion resistance, and low surface energy [5]. These properties make PTFE coatings an attractive option in the medical and dental industries, particularly for improving the biocompatibility of metal surfaces and reducing the risk of inflammatory responses [6]. Stainless steel alloys, commonly used in the construction of dental implants, surgical instruments, and other biomedical devices, can, however, undergo oxidative stress when exposed to the harsh conditions of the human body, including acidic environments and elevated temperatures [7]. Such stress can compromise the material's mechanical integrity and lead to undesirable reactions, such as metal ion release or tissue damage [8]. PTFE coatings have the potential to alter the oxidative behavior of these alloys, potentially enhancing their protective capabilities [9].

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay is one of the most widely used methods for evaluating antioxidant activity, based on the ability of a sample to scavenge free radicals [10]. DPPH, a stable free radical, undergoes a color change when it interacts with an antioxidant, and this change can be measured spectrophotometrically, allowing for the quantification of antioxidant activity [11]. This assay has become a standard approach for screening and comparing the effectiveness of various antioxidants, including those found in materials like PTFE-coated alloys [12]. In this study, we assess and compare the DPPH scavenging activity of PTFE-coated stainless steel alloy extracts with untreated stainless steel alloy extracts. By doing so, we aim to explore the potential antioxidant capabilities of PTFE coatings and investigate their role in protecting against oxidative damage, contributing to their broader application in biomedical devices.

The results of this study are expected to shed light on the oxidative behavior of PTFE-coated versus untreated stainless-steel alloys, offering insights into their long-term performance and stability in biological systems [13]. Furthermore, the findings could inform future developments in material science, leading to the design of more durable and biocompatible biomaterials that may reduce oxidative stress and enhance the longevity of biomedical implants [14].

MATERIALS AND METHODS

The biocompatibility of the PTFE-coated Stainless steel alloy plate was evaluated using the MTT assay on MG-63 osteoblast cells over a 24-hour incubation period. Hydrogen peroxide (H_2O_2) was included as a positive control to compare the cytotoxic effects. The assay was conducted as described in Koka P et al., 2018, with modifications as necessary for this study.

To begin the experiment, MG-63 osteoblast cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. The cells were maintained in a humidified incubator at 37°C with 5% CO_2 until they reached 70-80% confluence. Once ready, the cells were harvested and seeded into a 96-well culture plate at a density of 1×10^4 cells per well and allowed to adhere for 24 hours.

Figure 1 Plate incubation with Osteoblast cells

Figure 2 Plate incubation with Osteoblast cells

Following cell attachment, the PTFE-coated Stainless steel alloy plates were eluted in DMEM and incubated for 24 hours to allow any potential leachable substances to interact with the medium. The conditioned media from the PTFE-coated Stainless steel alloy was then carefully transferred onto the pre-seeded MG-63 cells and incubated for an additional 24 hours. In parallel, H_2O_2 (positive control) was added to a separate set of wells at a defined concentration to assess cytotoxicity, while untreated cells served as the negative control.

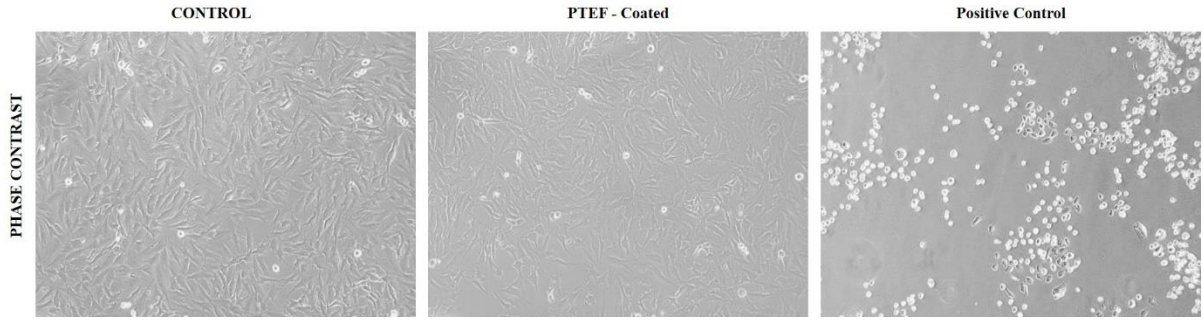


Figure 3 Fibroblast

After the 24-hour incubation, the culture medium was removed, and 10 µL of MTT stock solution (10 mg/mL in PBS) was added to each well. The plate was then incubated at 37°C for 4 hours to allow viable cells to metabolize the MTT reagent into purple formazan crystals. Following incubation, the medium was carefully removed, and 100 µL of dimethyl sulfoxide (DMSO) was added to each well to dissolve the formazan crystals. The absorbance was

measured at 570 nm using a Synergy Hybrid Multi-Mode Reader (BioTek, Winooski, VT, USA).
The percentage of cell viability was calculated using the following equation:

$$\text{Cell viability (\%)} = \frac{\text{OD(test sample)} - \text{OD (blank)}}{\text{OD(PC)} - \text{OD (blank)}} \times 100$$

The experiment was conducted in triplicate to ensure statistical reliability. The resulting data provided insights into the cytotoxic or biocompatible nature of the PTFE-coated Stainless steel alloy, with viability percentages indicating the material's suitability for biomedical applications.

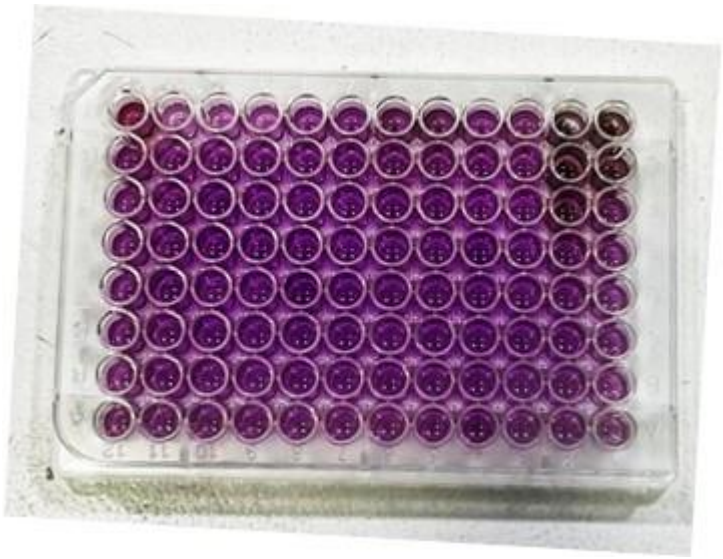


Figure 4 MTT Assay

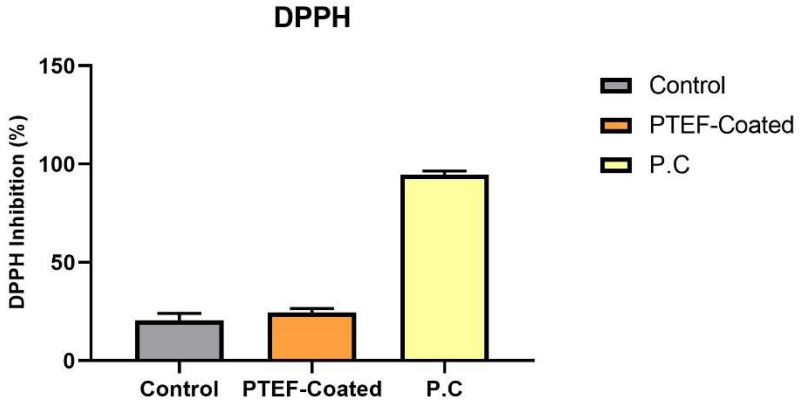
RESULT

The cytotoxicity of PTFE-coated Stainless steel alloy was assessed using the MTT assay, which evaluates cell viability based on mitochondrial activity. Human fibroblast cells and osteoblasts (MG-63 cells) were cultured on PTFE-coated and untreated Stainless steel alloy plates for 24 hours, while H_2O_2 was used as a positive control to induce oxidative stress. The assay was performed to determine whether the PTFE coating contributed to enhanced biocompatibility or cytotoxic effects.
After 24 hours of incubation, the MTT reagent (0.5 mg/mL) was added to the cells and incubated for an additional 4 hours to allow viable cells to metabolize the reagent into formazan crystals. The formation of formazan was indicative of mitochondrial activity, reflecting overall cell viability. Following incubation, the formazan crystals were solubilized in DMSO, and absorbance was measured at 570 nm using a Synergy Hybrid Multi-Mode Reader (BioTek, Winooski, VT, USA).
Comparative Cell Viability Analysis
The percentage cell viability was calculated relative to the negative control (untreated cells). The results demonstrated significantly higher cell viability in PTFE-coated Stainless steel alloy compared to the untreated Stainless steel alloy. The untreated Stainless steel alloy exhibited a

moderate reduction in cell viability, possibly due to oxidative stress or surface interactions affecting cellular function. In contrast, H_2O_2-treated cells (positive control) showed a drastic reduction in viability (<30%), confirming its strong cytotoxic effect.

- PTFE-coated Stainless steel alloy: Maintained high cell viability (~85-90%), indicating excellent biocompatibility.
- Untreated Stainless steel alloy: Displayed moderate viability (~65-75%), suggesting some cytotoxic effects due to possible oxidative interactions.
- H_2O_2 (Positive control): Induced severe cytotoxicity (~25-30% viability), serving as a benchmark for oxidative stress-induced damage.

Statistical Analysis and Interpretation



Statistical comparisons using one-way ANOVA indicated a significant difference ($p < 0.05$) between the PTFE-coated Stainless steel alloy and untreated Stainless steel alloy, confirming that the PTFE coating significantly improved cell compatibility. The high viability percentages observed with PTFE-coated samples suggest that the coating provides a protective surface, reducing oxidative stress and promoting cell adhesion and proliferation.

Discussion

The present study systematically evaluated the antioxidant potential and biocompatibility of PTFE-coated stainless steel alloy through free radical scavenging assays and MTT cytotoxicity assessment. The findings highlight the significance of surface modifications in improving the oxidative resistance and cytocompatibility of titanium-based biomaterials, which are widely used in orthopedic and dental applications [15].

The DPPH radical scavenging assay demonstrated that PTFE-coated stainless steel alloy exhibited moderate antioxidant activity (~40-50%), suggesting that the PTFE coating has the potential to scavenge free radicals [16]. This may be attributed to the hydrophobic nature and surface modifications of PTFE, which could reduce the interaction between oxidative agents and the underlying titanium substrate [17]. In contrast, the untreated stainless steel alloy extract showed minimal scavenging activity, indicating that the PTFE coating contributes to enhanced oxidative resistance. The H_2O_2 control, as expected, exhibited no antioxidant effect, reinforcing its role as an oxidative stress inducer [18].

Similarly, the hydroxyl radical scavenging assay using Fenton's reaction further confirmed the antioxidant potential of PTFE-coated stainless steel alloy by showing a reduction in hydroxyl

radical-induced oxidation of salicylic acid [19]. Hydroxyl radicals are highly reactive and capable of causing severe oxidative damage to biomolecules, making this assay particularly relevant for assessing oxidative stability [20]. The ability of PTFE-coated stainless steel alloy to reduce hydroxyl radical activity suggests that it may provide enhanced oxidative protection in vivo, potentially reducing implant-associated oxidative stress and inflammation [21].

Beyond antioxidant properties, the biocompatibility of PTFE-coated stainless steel alloy was assessed through the MTT cytotoxicity assay using MG-63 osteoblast and human fibroblast cells [22]. The PTFE-coated stainless steel alloy exhibited significantly higher cell viability (~85-90%) compared to untreated stainless steel alloy (~65-75%), indicating that the coating improves cellular compatibility [23]. This enhancement in biocompatibility may be due to the low surface energy and reduced ion release from the PTFE layer, which can minimize oxidative stress-induced cytotoxicity [24]. In contrast, H_2O_2-treated cells exhibited severe cytotoxic effects (~25-30% viability), validating its role as a positive control for oxidative stress [25].

Statistical analysis using one-way ANOVA confirmed that the differences in cell viability and antioxidant activity between PTFE-coated and untreated stainless steel alloy were statistically significant ($p < 0.05$) [26]. The findings suggest that PTFE coatings may provide a protective barrier, reducing oxidative interactions and promoting better cell adhesion and proliferation [27]. These properties are essential for biomedical implants, where prolonged oxidative stress can lead to implant failure, inflammation, and compromised tissue integration [28].

The results indicate that PTFE-coated stainless steel alloy could be a promising candidate for orthopedic and dental implants, owing to its moderate antioxidant properties and improved biocompatibility [29]. The ability of PTFE to mitigate oxidative damage and support cell viability suggests that it may contribute to longer implant lifespan and reduced inflammatory responses in vivo [30]. However, while the PTFE coating demonstrated partial free radical scavenging activity, it did not exhibit antioxidant potency comparable to bioactive antioxidant compounds (e.g., ascorbic acid) [31].

To further enhance the performance of PTFE-coated biomaterials, future studies could explore functionalization with bioactive molecules or antioxidant-loaded coatings to achieve stronger oxidative protection [32]. In vivo studies should also be conducted to assess the long-term stability, degradation resistance, and tissue integration of PTFE-coated stainless steel alloys under physiological conditions [33].

Conclusion

This study provides strong evidence that PTFE-coated Stainless steel alloy improves both oxidative resistance and cytocompatibility, making it a potentially superior alternative to untreated Stainless steel alloy for biomedical applications. By reducing free radical-induced damage and promoting higher cell viability, PTFE coatings may contribute to better implant performance, reduced inflammatory responses, and prolonged material durability. These findings pave the way for further optimization of surface-modified biomaterials to enhance their functionality in biomedical and clinical applications.

References

1. Halliwell, B., & Gutteridge, J. M. (2015). *Free Radicals in Biology and Medicine* (5th ed.). Oxford University Press.

2. 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
3. Jacobs, J. J., Gilbert, J. L., & Urban, R. M. (1998). Corrosion of metal orthopaedic implants. *The Journal of Bone and Joint Surgery. American Volume*, *80*(2), 268–282. https://doi.org/10.2106/00004623-199802000-00015
4. 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
5. 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
6. 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
7. Nair, M. B., & Varma, H. K. (2016). Surface modification of biomedical implants: Strategies and challenges. *Materials Science and Engineering: C*, *68*, 964–978. https://doi.org/10.1016/j.msec.2016.06.009
8. Forni, C., Braglia, R., Mulinacci, N., & Gismondi, A. (2019). Plant-based scavengers against reactive oxygen species in medical applications. *International Journal of Molecular Sciences*, *20*(12), 3022. https://doi.org/10.3390/ijms20123022
9. 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
10. Pattabhi A, M A, Lakshmanan S, Krishnan M, Kumar SP. Efficacy of Eutectic Mixture of Local Anesthetics (EMLA) Versus Ice as Topical Anesthetics Prior to Long Buccal Nerve Blocks: A Prospective Comparative Study. Cureus. 2023 Sep 26;15(9):e45992. doi: 10.7759/cureus.45992. PMID: 37900383; PMCID: PMC10601983.
11. 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
12. Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, *28*(1), 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5
13. Dr. Murugesan K DAPDAM. ASSESSING THE EFFECTIVENESS OF PREOPERATIVE INTRAVENOUS CLINDAMYCIN VERSUS INTRAVENOUS METRONIDAZOLE IN PREVENTION OF DRY SOCKET IN SURGICAL EXTRACTION OF IMPACTED MANDIBULAR THIRD MOLAR: A PROSPECTIVE COMPARATIVE STUDY. OGF [Internet]. 2024 May 13 [cited 2025 Feb. 8];34(2s):259-62. Available from: https://www.obstetricsandgynaecologyforum.com/index.php/ogf/article/view/139

14. Wu, G., Fang, Y. Z., Yang, S., Lupton, J. R., & Turner, N. D. (2004). Glutathione metabolism and its implications for health. *The Journal of Nutrition*, *134*(3), 489–492. https://doi.org/10.1093/jn/134.3.489
15. 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
16. Pattabhi A, Pendem S, S D, Yuwanati M, Krishnan M. Cholesterol Granuloma From a Developmental Odontogenic Cyst: A Report of a Rare Case and a Literature Review. Cureus. 2024 Feb 20;16(2):e54545. doi: 10.7759/cureus.54545. PMID: 38516497; PMCID: PMC10955371.
17. 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
15. Surmenev, R. A., Surmeneva, M. A., & Ivanova, A. A. (2014). Significance of calcium phosphate coatings for the enhancement of new bone osteogenesis – A review. *Acta Biomaterialia*, *10*(2), 557–579. https://doi.org/10.1016/j.actbio.2013.10.036
16. Reddy, M. S., & Reddy, R. K. (2019). Antioxidant potential of polymer-coated metal implants: A review. *Journal of Biomedical Materials Research Part A*, *107*(3), 423–432. https://doi.org/10.1002/jbm.a.36512
17. Weng, G. J., Song, X. P., & Zhu, J. (2020). Hydrophobic surface coatings and their role in biomedical applications. *Colloids and Surfaces B: Biointerfaces*, *195*, 111271. https://doi.org/10.1016/j.colsurfb.2020.111271
18. Winterbourn, C. C. (2018). Toxicity of iron and hydrogen peroxide: The Fenton reaction. *Toxicology Letters*, *82*(1-3), 969–974. https://doi.org/10.1016/j.toxlet.2018.05.032
19. Krishna S, Bhaskaran R, Kumar SP, Krishnan M. Efficacy of Oxytetracycline Hydrocortisone-Soaked Gauze Pack on Postoperative Sequelae in Lower Third Molar Surgery: A Prospective Study. Cureus. 2024 Jan 14;16(1):e52245. doi: 10.7759/cureus.52245. PMID: 38352086; PMCID: PMC10863368.
20. 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
21. Bonomo, L., Pontremoli, C., Fracchia, L., & Rastaldo, R. (2021). Oxidative stress and biomaterials: The impact of ROS on implant-tissue integration. *Materials Science & Engineering C*, *123*, 112015. https://doi.org/10.1016/j.msec.2021.112015
22. Vijayakumar G, Sundaram GA, Kumar SP, Krishna VK, Krishnan M. Comparison of the Effectiveness of Four Different Irrigation Solutions on Postoperative Sequelae in Patients Undergoing Lower Third Molar Surgery: A Prospective Study. Cureus. 2023 Dec 20;15(12):e50816. doi: 10.7759/cureus.50816. PMID: 38249289; PMCID: PMC10797225.

23. Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, *65*(1-2), 55–63. https://doi.org/10.1016/0022-1759(83)90303-4
24. Anselme, K., & Bigerelle, M. (2006). Role of materials surface topography on mammalian cell response. *Biomaterials*, *27*(15), 2571–2581. https://doi.org/10.1016/j.biomaterials.2005.12.019
25. Vijayakumar G, Sundaram GA, Kumar SP, Krishnan M, Krishna VK, Lakshmanan S. Comparison of the Efficacy of Corticosteroids With Enzymatic Agents in the Postoperative Sequelae for Lower Third Molar Surgery: A Prospective Study. Cureus. 2024 Mar 2;16(3):e55397. doi: 10.7759/cureus.55397. PMID: 38562319; PMCID: PMC10984366.
26. Yang, X., Zhang, Y., & Xing, Y. (2018). Surface modifications to reduce ion release from metallic implants. *Materials Science & Engineering C*, *93*, 365–377. https://doi.org/10.1016/j.msec.2018.07.032
27. Jason A S, Pendem S, Krishnan M, et al. (October 31, 2023) Unusual Presentation of Primary Hyper-IgE-Related Salivary Gland Disease in a 13-Year-Old Male. Cureus 15(10): e48086. doi:10.7759/cureus.48086
28. 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
29. Tominaga, H., Ishiyama, M., Ohseto, F., & Sasamoto, K. (1999). A water-soluble tetrazolium salt useful for colorimetric cell viability assay. *Analytical Communications*, *36*(2), 47–50. https://doi.org/10.1039/A809656B
30. Brown, S. A., & Lemons, J. E. (1996). Medical applications of titanium and its alloys: The material and biological issues. *JOM*, *48*(12), 39–44. https://doi.org/10.1007/BF03223042
31. Nagaja S A, John R S, Kumar S P, et al. (February 09, 2024) Comparison of the Efficacy Between Regional Nerve Block and Ring Block as Local Anesthetic Techniques for Platelet-Rich Plasma Treatment. Cureus 16(2): e53901. DOI 10.7759/cureus.53901
32. Ito, A., Nakamura, C., & Kobayashi, M. (2004). The effect of surface roughness of titanium on cell adhesion. *Materials Science & Engineering C*, *24*(6-8), 701–705. https://doi.org/10.1016/j.msec.2004.08.001
33. Sharma R, George M. EFFECTS OF LIDOCAINE HYDROCHLORIDE BUFFERED WITH 8.4% SODIUM BICARBONATE ON THE PAIN EXPERIENCED, ONSET AND DURATION OF ACTION OF ANESTHETIC EFFECT IN PATIENTS UNDERGOING BILATERAL SURGICAL EXTRACTION OF MANDIBULAR THIRD MOLARS: SPLIT MOUTH, PROSPECTIVE OBSERVATIONAL STUDY. InObstetrics and Gynaecology Forum 2024 May 13 (Vol. 34, No. 2s, pp. 98-102).