

CYTOTOXICITY STUDY OF BIOMAGNETIC MEMBRANES WITH POTENTIAL APPLICATION IN GUIDED TISSUE REGENERATION

**Andrei Mihai Gavrilovici¹, Mihai MC Fabricky^{#1}, Marius Ciprian Manole ^{*2},
Dan Onisei^{*3}, Daliborca Cristina Vlad⁴, Ioan Bica⁵, Meda-Lavinia Negruțiu^{6,7}**

¹ Department of Prosthodontics, Faculty of Dental Medicine, University of Medicine and Pharmacy "Victor Babeș" Timișoara

²Department of Prosthetic Dentistry and Dental Materials, Faculty of Dental Medicine, "Iuliu Hațieganu" University of Medicine and Pharmacy Cluj-Napoca, Romania

³ Department of Prosthodontics, Faculty of Dental Medicine, University of Medicine and Pharmacy "Victor Babeș" Timișoara

⁴ Pharmacology Department, Faculty of Medicine, "Victor Babeș" University of Medicine and Pharmacy Timișoara, Romania

⁵ Faculty of Physics, West University of Timisoara, Timisoara, Romania

⁶ Department of Prostheses Technology and Dental Materials, Faculty of Dentistry, University of Medicine and Pharmacy "Victor Babeș" Timișoara

⁷ Research Center in Dental Medicine Using Conventional and Alternative Technologies, "Victor Babeș" University of Medicine and Pharmacy Timișoara, Romania

#Authors with equal contribution with the first author: Mihai MC Fabricky

*Corresponding authors: **Marius Ciprian Manole**, e-mail marius.manole@umfcluj.ro
Dan Onisei

ABSTRACT

This study evaluates the cytotoxic effects of biomagnetic membranes on healthy human cells, with an emphasis on their suitability for use in guided tissue regeneration (GTR). The aim of this study was to evaluate the biocompatibility of impregnation products used in bioactive magnetic membranes on human fibroblasts. Fibroblasts were selected due to their essential role in the maintenance and remodeling of the extracellular matrix (ECM), where they contribute to the synthesis of critical structural components, particularly collagen fibers. These cells also influence cellular signaling pathways involved in tissue repair and regeneration. The biocompatibility of materials intended for use in GTR techniques is of paramount importance, as any cytotoxic effects could impair fibroblast function and compromise healing outcomes. Given the emerging interest in magnetically responsive biomaterials for controlled therapeutic applications, this study focused on determining whether the impregnated magnetizable membranes support fibroblast viability and could therefore serve as promising scaffolds for regenerative medicine. Using in vitro assays, the biocompatibility of the membranes was assessed, revealing minimal cytotoxicity and high potential for clinical application in regenerative medicine.

Key words: biomagnetic membranes, guided tissue regeneration, cytotoxicity

INTRODUCTION

The development of active biomagnetic membranes, which can function both as physical barriers and as stimulatory agents in guided bone regeneration (GBR) procedures, represents a promising avenue in regenerative medicine. These membranes may offer dual functionality by not only preventing soft tissue invasion into bone defects but also by actively promoting osteogenesis through magnetically induced cellular

stimulation. Investigating the conditions that enhance rapid neo-bone formation is particularly relevant for improving outcomes in dedicated surgical techniques within the oro-maxillofacial field. Such advancements could lead to more predictable and accelerated healing in complex clinical scenarios requiring bone regeneration [1-3].

Composite membranes are complex structures composed of two or

more materials arranged in a spatial configuration where one dimension is significantly smaller than the others. The constituent materials exhibit markedly different chemical and physical properties. One of these components, referred to as the reinforcement, acts as a matrix that maintains the relative positioning of the others. The physical and mechanical properties of the resulting membrane are generally enhanced compared to those of the individual components. This improvement is primarily due to the reinforcement, which contributes to strengthening the matrix and optimizing its functional performance [4-6].

The increasing demands of modern society require the development of new types of membranes whose properties can be remotely controlled [7]. In this context, biomagnetic active membranes represent promising candidates. Specifically, our study focused on the production of membranes based on poly(DL-lactide), impregnated with apitherapeutic products, bone particles, and a magnetizable phase composed of iron carbonyl and silver nano/microparticles. We also investigated the cytotoxicity of these multifunctional systems. Honey, a natural substance widely recognized for its therapeutic properties—particularly its anti-inflammatory and antiseptic effects—is extensively used in various apicultural products [8].

Iron carbonyl, on the other hand, is a biodegradable industrial compound commercially available as a fine powder. It is frequently employed in the development of novel materials, such as magnetorheological suspensions [9] and elastomers [10], and has shown potential in biomedical applications [4-6].

Silver powder in the form of nano- and microparticles is also biodegradable and plays a crucial role in the development of layers that absorb electromagnetic radiation [11]. In the magnetizable membranes investigated in this study, silver microparticles contribute to

establishing predefined electrical conductivity and help create a local environment with anti-inflammatory and antimicrobial properties. Compared to other magnetizable membranes, the incorporation of silver nanoparticles enhances the overall performance of the composite, making it more suitable for various biomedical applications, including dermatological and dental treatments [12].

The primary objective of this study was to evaluate the biocompatibility of the impregnation components used in bioactive magnetic membranes on human fibroblasts. These cells are integral to the extracellular matrix and are responsible for the synthesis of matrix components, including collagen fibers. Their activity supports connective tissue cell differentiation and tissue regeneration, making them an appropriate *in vitro* model for assessing cytocompatibility in the context of guided tissue regeneration strategies.

MATERIALS AND METHODS

In this study, we evaluated the viability and proliferation of human fibroblasts in contact with impregnation products derived from bioactive magnetic membranes. The investigation was carried out through *in vitro* assays using the hdfa human dermal fibroblast cell line (gibco). reagents and consumables used for cell culture were sourced from invitrogen, gibco, and thermo scientific.

Ethanol extraction was performed on the products of interest. Following solvent evaporation, the extracts were resuspended in dimethyl sulfoxide (DMSO) for use in biological testing. A comparative analysis was conducted on alcoholic extracts from the following formulations:

- E1 – extract from a product composed of 75% honey and 25% propolis

- E2 – extract from a product composed of 75% honey and 25% Fe₂O₃ nanoparticles
- E3 – extract from a product composed of 75% honey and 25% graphene nanoplatelets
- E4 – extract from a product composed of 75% honey and 25% silver nanoparticles
- E5 – extract from a product composed of 75% honey and 25% iron carbonyl powder

These formulations were selected to investigate the influence of different functional additives on fibroblast behavior, with a focus on their cytocompatibility and regenerative potential.

The honey used in this study was a 100% natural linden (*Tilia* spp.) honey, known for its strong antimicrobial and anti-inflammatory properties. It was sourced from apiaries located in linden forest regions. The honey had a mass density of $\rho_h = 1.496 \text{ g/cm}^3$ at 22°C.

Ferric oxide (Fe₂O₃) nanoparticles were purchased from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany), product code 544884. The powder had a minimum purity of 99.9%, a particle size of less than 50 nm, and a specific surface area ranging from 50 to 245 m²/g.

Graphene nanoplatelets were obtained from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany), product code 900407. These particles had a reported purity of 100%, particle diameters smaller than 2 μm , and a thickness of a few nanometers. The specific surface area was approximately 750 m²/g, and the bulk density was $\rho^G = 0.2\text{--}0.4 \text{ g/cm}^3$ at 22°C.

Silver microparticles were purchased from Sigma-Aldrich (Saint Louis, Missouri, USA), product code 327085, with a minimum purity of 99.9%. The particles were spherical in shape, with an average diameter of $d_{ag} \approx 3 \text{ }\mu\text{m}$, and a bulk density of $\rho_{ag} = 1.336 \text{ g/cm}^3$ at 22°C.

Carbonyl iron (CI) microparticles were purchased from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany),

product code C-3518. The powder had a minimum purity of 99.7%, with spherical particles averaging a diameter of $d(\text{CI}) \approx 5 \text{ }\mu\text{m}$. The bulk density was $\rho(\text{CI}) = 3.512 \text{ g/cm}^3$ at 22°C.

Cell Culture Conditions

Human dermal fibroblast cells (HDFa, Gibco) were thawed and seeded into 75 cm² culture flasks for expansion. Cells were cultured in a specific growth medium consisting of Dulbecco's Modified Eagle Medium (DMEM) supplemented with 1% antibiotic/antimycotic solution and 10% fetal bovine serum (FBS), maintained at 37°C in a 5% CO₂ atmosphere. Upon reaching 80% confluence, the cells were washed with phosphate-buffered saline (PBS) and detached using trypsinization.

Cell Proliferation Assay (MTT Test)

Cell proliferation was assessed using the MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide). Cells were seeded in 96-well plates at a density of 3×10^5 cells/well in 100 μL of culture medium. After 24 hours (to allow for cell adhesion), test solutions consisting of 1% extract (v/v) dissolved in DMSO and culture medium were added. Incubation continued for an additional 24 and 72 hours.

The control group (100% growth reference) consisted of cells cultured in the same specific medium containing only the DMSO vehicle, without any added extract. After incubation, the culture medium was replaced with fresh medium containing MTT (5 mg/mL in PBS, pH 7.4). Cells were incubated at 37°C in the dark. After 3 hours, the resulting insoluble formazan crystals were dissolved in DMSO, and absorbance was measured at 570 nm (reference wavelength: 630 nm) using a Tecan microplate reader.

Each sample was analyzed in triplicate, and results were expressed as a percentage relative to the control. Cell

proliferation (CP, %) was calculated using the formula:

$$CP (\%) = [(A_{Test}) / (A_{Control})] \times 100,$$

where A_{Test} is the absorbance measured at 570 nm.

Cell Morphology Analysis

Cell morphology was observed under a Nikon Eclipse U2000 microscope. Images were captured using an integrated digital camera system.

RESULTS

Microscopic examination of fibroblasts incubated with the test products demonstrated that the cells retained their characteristic spindle-shaped morphology, indicative of a healthy and unaltered phenotype. No morphological abnormalities or signs of cytotoxicity were observed. Furthermore, the cellular density remained comparable to that of the control group across all experimental conditions at the 24-hour time point, suggesting that the tested compounds did not adversely affect cell proliferation or viability (Figures 1-2)

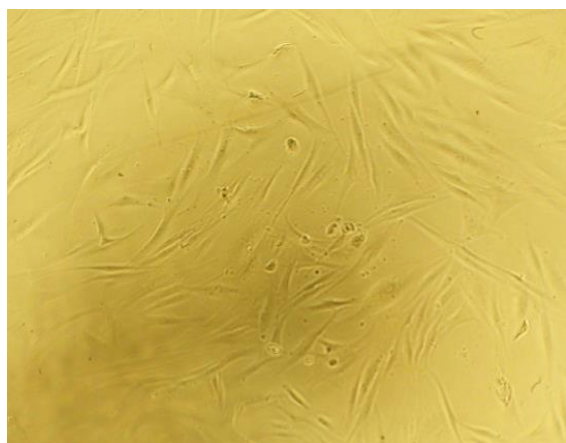


Figure 1. Representative image of fibroblast morphology in the control group after 24 hours of incubation. Cells exhibit the characteristic spindle-shaped morphology. Microculture flask (MCF), 200× magnification.

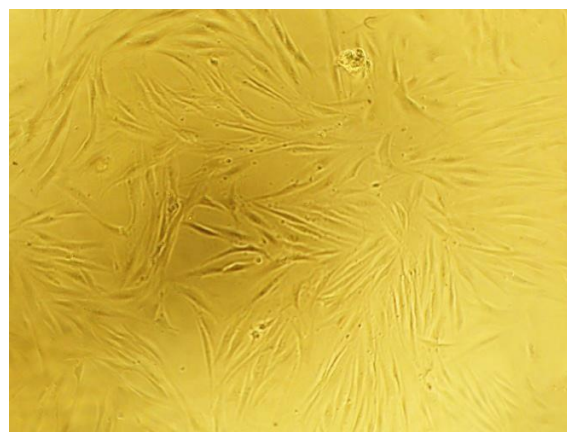


Figure 2. Representative image of fibroblast morphology after 24 hours of incubation with E3. Cells maintained their characteristic spindle-shaped appearance. Microculture flask (MCF), 200× magnification.

Microscopic analysis performed at 72 hours post-incubation further confirmed that fibroblasts exposed to the tested products maintained normal morphology and viability. Cell density across all experimental groups remained comparable to that of the control, with no detectable signs of cytotoxicity. The fibroblasts retained their characteristic spindle-shaped morphology, indicating preserved cellular integrity and typical phenotypic features throughout the observation period (Figures 3-4)



Figure 3. Representative image of fibroblast morphology in the control group after 72 hours of incubation. Cells exhibit the typical spindle-shaped morphology. Microculture flask (MCF), 100× magnification.

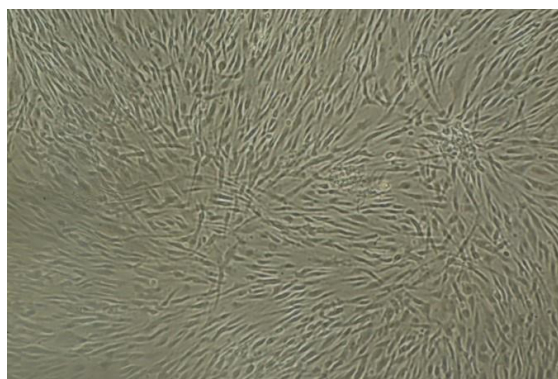


Figure 4. Representative image of fibroblast morphology after 72 hours of incubation with E2. No morphological changes observed; cells retained their characteristic spindle shape. Microculture flask (MCF), 100× magnification.

Regarding cell proliferation, as assessed by the MTT assay, no significant differences were observed between the experimental groups and the control group, which was considered to represent 100% proliferation (Table 1). The MTT assay (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) is based on the conversion of MTT into formazan crystals by metabolically active cells, reflecting mitochondrial activity. Since total mitochondrial activity correlates with the number of viable cells in most cell populations, this assay is widely used to evaluate the in vitro cytotoxic effects of various substances on cell lines [13].

GROUP	CELL PROLIFERATION (%)	
	24h	72h
control	100%	100%
E1	88.8601%	87.82435%
E2	97.95918%	91.11111%
E3	104.0179%	118.4035%
E4	99.28469%	114.3258%
E5	96.10951%	88.86159%

Table 1. Cell proliferation assessed by the MTT assay.

Notably, after 72 hours of exposure, compounds E3 (an extract from a product composed of 75% honey and 25% graphene nanoplatelets) and E4 (an extract from a product composed of 75%

honey and 25% silver nanoparticles) showed a stimulatory effect on cell proliferation, as evidenced by increased mitochondrial activity (Figure 5). These findings suggest a potential proliferative or bioactive influence of E3 and E4 on fibroblasts under the conditions tested.

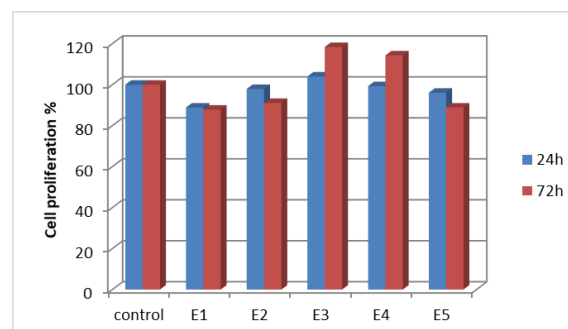


Figure 5. Graphical representation of cell proliferation at 24 and 72 hours.

DISCUSSIONS

The development of membranes based on magnetizable nano- and microparticles as devices for use in oral surgery represents a current and promising research direction. This approach relies on the use of nano- and microparticles assembled in various media, including liquids and fibers derived from natural and/or synthetic polymers [14-16]. One of the main challenges faced by researchers in dental medicine is the creation of materials that can withstand the harsh conditions of the oral environment while remaining biologically stable and biocompatible [17, 18]. Nano- and microparticles are gaining increasing attention in dentistry due to their unique physicochemical and biological properties, including biocompatibility, size, charge, high surface area, strength, solubility, chemical and surface reactivity, color, high stability, and thermal conductivity [19-21]. These characteristics have enabled the development of innovative materials and the enhancement and diversification of their functions. Despite their numerous advantages, some nano- and microparticles also present limitations, such as potential

toxicity, restricted delivery capabilities, and handling challenges [18].

Relatively recent studies [22] have demonstrated that carbonyl iron tablets are better tolerated by the human body compared to ferrous sulfate tablets. Furthermore, these studies highlight that iron ions resulting from biochemical reactions help replenish the body's iron reserves and may also contribute to the prevention of certain mental health disorders [22]. For this reason, carbonyl iron microparticles were selected for the fabrication of the magnetizable membranes in this study.

Silver nanoparticles exhibit several attractive properties, including excellent electrical conductivity, chemical stability, and notable antifungal and bactericidal effects. As a result, silver nanoparticles have found a wide range of biomedical applications [23-25].

Silver nanoparticles are well known for their remarkable antimicrobial properties against a wide range of pathogens, including bacteria, fungi, and viruses. However, the exact mechanisms responsible for their bactericidal effects remain unclear. There is ongoing debate as to whether it is the silver nanoparticles themselves or the silver ions they release that exert cytotoxic effects on microorganisms [26]. It has been shown that silver nanoparticles are effective against both Gram-negative and Gram-positive bacterial strains [27]. With the increasing biomedical applications of silver nanoparticles, there is a corresponding rise in human cell exposure, raising concerns about their potential cytotoxicity [28].

Therefore, the development of silver nanoparticles and their nanocomposites with strong antimicrobial properties but minimal cytotoxicity to host cells is crucial for the treatment of bacterial infections. Similar to the findings of this study, several reports in the scientific literature have shown that silver nanoparticles are non-toxic to mouse

fibroblasts and may even stimulate their proliferation [29-31].

Wound healing is a complex process involving numerous interdependent immunological and pathophysiological mediators that act to restore the cellular integrity of damaged tissue. The process includes four distinct but overlapping phases: inflammation, proliferation/regeneration, and tissue fibroplasia [32].

With the emergence of drug-resistant bacteria, many conventional antimicrobial agents have become ineffective for wound treatment, and failures of standard therapies have been increasingly reported. As a result, alternative therapies are being explored — among them, the use of honey as a wound healing agent. Honey has recently gained clinical interest for its potential use in wound care and regenerative medicine [33, 34].

Scaffold systems have been developed to ensure the controlled and sustained release of apitherapeutic compounds while maintaining exudate absorption at the wound site. These scaffolds, developed through tissue engineering, primarily include electrospun fibers, cryogels, and hydrogels [35]. Such delivery platforms can enhance the targeted release of active substances in wound management applications [33,34].

CONCLUSIONS

The present study confirmed that biomagnetic membranes incorporating apitherapeutic products, graphene nanoplatelets, carbonyl iron microparticles, and silver nanoparticles exhibit no cytotoxicity and support fibroblast proliferation *in vitro*. these findings underscore the biocompatibility of the developed composite membranes and their potential suitability for biomedical

applications, particularly in oral surgery and tissue engineering.

Moreover, by adjusting the membrane composition and magnetic induction parameters, it is possible to precisely control the electric current intensity within the device. This enables the generation of a localized thermal field, which may enhance the diffusion of bioactive compounds from the membrane to the target tissue. Such a mechanism is

especially advantageous in guided tissue regeneration, where both controlled drug delivery and biointegration are critical.

Overall, the multifunctional properties of these engineered membranes suggest a promising platform for future development of smart, responsive biomedical devices. Further in vivo studies are necessary to validate these results and to optimize their performance in clinical settings.

Acknowledgements

This work was sustained by the "Victor Babes" University of Medicine and Pharmacy of Timișoara, Romania.

Conflicts of Interest: The authors declare no conflict of interest.

REFERENCES

1. Isler SC, Soysal F, Ceyhanli F, Bakirarar B, Unsal B, Regenerative surgical treatment of peri-implantitis using either a collagen membrane or concentrated growth factor: A 12-month randomized clinical trial, *Clinical Implant Dentistry and Related Research*, 2018, 20(5):703–712
2. Trobos M, Juhlin A, Shah FA, Hoffman M, Sahlin H, Dahlin C, In vitro evaluation of barrier function against oral bacteria of dense and expanded polytetrafluoroethylene (PTFE) membranes for guided bone regeneration, *Clinical Implant Dentistry and Related Research*, 2018, 20(5):738–748
3. Makowiecki A, Botzenhart U, Seeliger J, Heinemann F, Biocev P, Dominiak M, A comparative study of the effectiveness of early and delayed loading of short tissue-level dental implants with hydrophilic surfaces placed in the posterior section of the mandible—A preliminary study, *Annals of Anatomy*, 2017, 212:61–68
4. Bica I, Anitas EM, Sfirloaga P. Magnetizable Membranes Based on Cotton Microfibers, Honey, Carbonyl Iron, and Silver Nanoparticles: Effects of Static Magnetic Fields and Medium-Frequency Electric Fields on Electrical Properties. *Magnetochemistry*. 2023; 9(1):19
5. Bica I, Bunoiu OM. Magnetorheological Hybrid Elastomers Based on Silicone Rubber and Magnetorheological Suspensions with Graphene Nanoparticles: Effects of the Magnetic Field on the Relative Dielectric Permittivity and Electric Conductivity. *Int J Mol Sci*. 2019;20(17):4201.
6. Gavrilovici, A. M., Anitas, E. M., Chirigiu, L., Bica, I., Negrutiu, M. L., Magnetodielectric Effects in Magnetorheological Elastomers Based on Polymer Fabric, Silicone Rubber, and Magnetorheological Suspension, *Advances in Polymer Technology*, 2019, 1983547, 5 pages. <https://doi.org/10.1155/2019/1983547>
7. Elmahaisi, M.F.; Azis, R.S.; Ismail, I.; Muhammad, F.D. A review on electromagnetic microwave absorption properties: Their materials and performance. *J. Mater. Res. Technol.* 2022, 20:2188–2220
8. Cianciosi, D.; Forbes-Hernández, T.Y.; Afrin, S.; Gasparini, M.; Reboredo-Rodriguez, P.; Manna, P.P.; Zhang, J.; Bravo, L.L.; Martín F. S.; Agudo, T.P.; et al. Phenolic Compounds in Honey and Their Associated Health Benefits: A Review. *Molecules* 2018, 23:2322.

9. Plachy, T.; Cvek, M.; Munstr, L.; Hanulikova, B.; Suly, P.; Vesel, A.; Cheng, Q. Enhanced magnetorheological effect of suspensions based on carbonyl iron particles coated with poly (amidoamine) dendrons. *Rheol. Acta* 2021, 60, 263–276
10. Salem, A.M.H.; Ali, A.; Ramli, R.B.; Muthalif, A.G.A.; Julai, S. Effect of Carbonyl Iron Particle Types on the Structure and Performance of Magnetorheological Elastomers: A Frequency and Strain Dependent Study. *Polymers* 2022, 14, 4193
11. Zaharia, C.; Duma, V.-F.; Sinescu, C.; Socoliuc, V.; Craciunescu, I.; Turcu, R.P.; Marin, C.N.; Tudor, A.; Rominu, M.; Negrutiu, M.-L. Dental Adhesive Interfaces Reinforced with Magnetic Nanoparticles: Evaluation and Modeling with Micro-CT versus Optical Microscopy. *Materials* 2020, 13, 3908. <https://doi.org/10.3390/ma13183908>
12. Bruna, T.; Maldonado-Bravo, F.; Jara, P.; Caro, N. Silver Nanoparticles and Their Antibacterial Applications. *Int. J. Mol. Sci.* 2021, 22:7202
13. Ghasemi M, Turnbull T, Sebastian S, Kempson I. The MTT Assay: Utility, Limitations, Pitfalls, and Interpretation in Bulk and Single-Cell Analysis. *Int J Mol Sci.* 2021;22(23):12827
14. Zaharia, C; Sinescu, C; Gabor, AG; Cojocariu, AC; Socoliuc, V; Szuhaneck, C; Negrutiu, ML, MicroCT Analisis of Dental Adhesives Loaded with Nanoparticles, *Rev.Chim-Bucharest*, 2019,70(5):1769-1773
15. Topala, F; Nica, LM; Boariu, M; Negrutiu, ML; Sinescu, C; Marinescu, A; Cirligeriu, LE; Stratul, SI; Rusu, D; Chincia, R; Duma, VF; Podoleanu, A, En-face optical coherence tomography analysis of gold and silver nanoparticles in endodontic irrigating solutions: An in vitro study, *Exp. Ther. Med.*, 2021, 22(3):992.
16. Zaharia C., Sinescu C., Gabor A.G., Socoliuc V., Talpos S, Hajaj T., Sfirloaga P., Oancea R., Miclau M., Negrutiu M.L., Influences of Polymeric Magnetic Encapsulated Nanoparticles on the Adhesive Layer for Composite Materials Used for Class I Dental Fillings, *Mat.Plast.*, 2019, 56(2):399-404.
17. Meena A, Mali H, Patnaik A, Kumar S. Comparative investigation of physical, mechanical and thermomechanical characterization of dental composite filled with nanohydroxyapatite and mineral trioxide aggregate. *e-Polymers.* 2017;17(4): 311-319
18. Omid, M.; Fatehinya, A.; Farahani, M.; Akbari, Z.; Shahmoradi, S.; Yazdian, F.; Tahriri, M.; Moharamzadeh, K.; Tayebi, L.; Vashae, D. *Characterization of Biomaterials; Biomaterials for Oral and Dental Tissue Engineering*, Elsevier: Singapore, 2018, 97–115
19. Makkar H, Patri G. Fabrication and Appraisal of Poly (Lactic-Co-Glycolic Acid) - Moxifloxacin Nanoparticles using Vitamin E-TPGS: A Potential Intracanal Drug Delivery Agent. *J Clin Diagn Res.* 2017;11(6):ZC05-ZC08
20. Tanaka M, Okinaga T, Iwanaga K, et al. Anticancer effect of novel platinum nanocomposite beads on oral squamous cell carcinoma cells. *J Biomed Mater Res B Appl Biomater.* 2019;107(7):2281-2287
21. Ketabat F, Pundir M, Mohabatpour F, et al. Controlled Drug Delivery Systems for Oral Cancer Treatment-Current Status and Future Perspectives. *Pharmaceutics.* 2019;11(7):302
22. Ahmed TN, Shahriar I, Md Iqbal JU, Rowshan MM, Yusuf R. Comparison of Efficacy and Tolerability of Ferrous Sulfate and Carbonyl Iron in Iron Deficiency Anemia in Pregnant Woman. *Sir Salimullah Med. Coll. J. [Internet].* 2022 Apr. 27 [cited 2023 Aug. 29];30(1):83-8.
23. Dakal T.C., Kumal N., Majumdal R., Yadav V. Mechanistic basis of antimicrobial actions of silver nanoparticles. *Front. Microbiol.* 2016; 7:1831
24. D'Agostino A., Taglietti A., Desando R., Bini M., Patrini M., Dacarro G., Cucca L., Pallavicini P., Grisoli P. Bulk surfaces coated with triangular silver nanoplates: Antibacterial action based on silver release and photo-thermal effect. *Nanomaterials.* 2017; 7:7
25. Burdusel A.C., Gherasim O., Grumezescu A.M., Mogoanta L., Fica A., Andronescu E. Biomedical applications of silver nanoparticles: An up-to-date overview. *Nanomaterials.* 2018;8:681

26. Barros C.H., Fulaz S., Stanisic D., Tasic L. Biogenic nanosilver against multidrug-resistant bacteria (MRDB) Antibiotics. 2018; 7:69
27. Taglietti A., Fernandez Y.A., Amato E., Cucca L., Dacarro G., Grisoli P., Necchi V., Pallavicini P., Pasotti L., Patrini M. Antibacterial activity of glutathione-coated silver nanoparticles against gram positive and gram negative bacteria. *Langmuir*. 2012; 28:8140–8148
28. Akter M., Sikder M.T., Rahman M., Ullah A.K., Hossain K.F., Banik S., Hosokawa T., Saito T., Kurasaki M. A systematic review on silver nanoparticles-induced cytotoxicity: Physicochemical properties and perspectives. *J. Adv. Res.* 2018; 9:1–16
29. Alarcon E.I., Vulesevic B., Argawal A., Ross A., Bejjani P., Podrebarac J., Ravichandran R., Phopase J., Suuronen E.J., Griffith M. Coloured cornea replacements with anti-infective properties: Expanding the safe use of silver nanoparticles in regenerative medicine. *Nanoscale*. 2016;8:6484–6489
30. Liu X., Lee P.Y., Ho C.M., Lui V.C., Chen Y., Chi C.M., Tam P.K., Wong K.Y. Silver nanoparticles mediate differential responses in keratinocytes and fibroblasts during skin wound healing. *ChemMedChem*. 2010; 5:468–475
31. Liao C, Li Y, Tjong SC. Bactericidal and Cytotoxic Properties of Silver Nanoparticles. *Int J Mol Sci*. 2019; 20(2):449
32. Guo S, Dipietro LA. Factors affecting wound healing. *J Dent Res*. 2010; 89(3):219–29
33. Hixon KR, Klein RC, Eberlin CT, Linder HR, Ona WJ, Gonzalez H, et al. A critical review and perspective of honey in tissue engineering and clinical wound healing. *Adv Wound Care*. 2019;8(8):403–15
34. Hixon KR, Lu T, Carletta MN, McBride-Gagyi SH, Janowiak BE, Sell SA. A preliminary in vitro evaluation of the bioactive potential of cryogel scaffolds incorporated with Manuka honey for the treatment of chronic bone infections. *J Biomed Mater Res B Appl Biomater*. 2018;106(5):1918–33
35. Kalaf EG, Hixon KR, Kadakia PU, Dunn AJ, Sell SA. Electrospun biomaterials for dermal regeneration. *Electrospun materials for tissue engineering and biomedical applications*. UK/England: Woodhead Publishing; 2017. p. 179–231