Heliyon 9 (2023) e20932

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Evaluation of the biocompatibility, antibacterial and anticancer effects of a novel nano-structured Fe–Mn-based biodegradable alloys in-vitro study

Samir Ali Elborolosy [a], Lamis Ahmed Hussein [b], Hamada Mahran [c], Hany R. Ammar [d], S. Sivasankaran [d], Safa Fathy Abd El-Ghani [e,f], Mohamed Yehia Abdelfattah [g,*], Ahmed Wael Abou-Zeid [h,i], Shereen Hafez Ibrahim [j], Mohamed Mostafa Elshamaa [a]

[a] Oral and Maxillofacial Surgery Department, Faculty of Dentistry, Beni-Suef University, Beni-Suef, Egypt
[b] Dental Biomaterials, Department of Removable Prosthetics, Faculty of Dentistry, Misr International University, Cairo, Egypt
[c] Maxillofacial Surgery, General Surgery Department, Faculty of Medicine, Assiut University, Egypt
[d] College of Engineering, Mechanical Engineering Department, Qassim University, Buraidah, 51452, Saudi Arabia
[e] Oral and Maxillofacial Pathology Department, Faculty of Dentistry, Cairo University, Cairo, Egypt
[f] Oral Pathology Department, School of Dentistry, Newgiza University, Egypt
[g] Oral Biology Department, Faculty of Dentistry, Beni-Suef University, Beni-Suef, Egypt
[h] Department of Basic Dental Science, National Research Centre, Egypt
[i] Oral Biology Department, School of Dentistry, Newgiza University, Egypt
[j] Department of Conservative Dentistry, Faculty of Dentistry, Cairo University, Cairo, Egypt

ARTICLE INFO

Keywords:
Biocompatibility
Antibacterial activity
Anticancer effect
Novel nanostructured Fe–Mn
In-vitro study
Bone regeneration
Biodegradable alloys

ABSTRACT

Scientific backgrounds: Development of nanostructured biodegradable alloys has generated a great deal of interest in the recent years as they offer promising bioactive materials for reconstruction of bony defects following traumatic fractures or surgical excision of tumors.
Objectives: The aim of the current study was to investigate the biocompatibility of Iron–Manganese -based alloys (Fe–Mn) with addition of copper (Cu), Tungsten (W) and cobalt (Co) to obtain 3 different alloys namely, Fe–Mn–Cu, Fe–Mn–W, and Fe–Mn–Co on normal oral epithelial cell line,and their possible anticancer effect on MG-63: osteosarcoma cell line.
Materials and methods: The sulforhodamine B (SRB) assay was used to assess cell viability percentage of both cell lines after exposure to discs of the proposed experimental alloys. Moreover, the antibacterial effect of such alloys against *Escherichia coli (E. coli)* was tested using disc diffusion susceptibility (Kirby-Bauer method) and colony suspension method.
Results: The cell viability percentage of oral epithelial cell line showed a significant increase in all the experimental groups in comparison to the control group. The highest percentage was observed in Fe–Mn–Co group, followed by Fe–Mn–W then Fe–Mn–Cu, at 24 and 72-h intervals, respectively. While the cell viability percentage of osteosarcoma cell line showed significant increase in all the experimental groups at 24-h intervals, it showed a significant drop in all the study groups at 72-h intervals. The lowest percentage was observed in Fe–Mn–Cu group, followed by Fe–Mn–W then Fe–Mn–Co. Moreover, all the examined study groups didn't show any inhibition zones against *E. coli* reference culture.

* Corresponding author. Oral Biology Department, Faculty of Dentistry, Beni-Suef University, Beni-Suef, Egypt.
E-mail address: mohamed.yehia@ngu.edu.eg (M.Y. Abdelfattah).

https://doi.org/10.1016/j.heliyon.2023.e20932
Received 30 March 2023; Received in revised form 22 September 2023; Accepted 11 October 2023
Available online 14 October 2023

Conclusions: The novel nanostructured biodegradable Fe–Mn–Cu, Fe–Mn–W, and Fe–Mn–Co metal alloys exhibit good biocompatibility on oral epithelial cell lines with the enhancement of cell proliferation in a time-dependent manner that favors bone regeneration. On the other hand, all the alloys manifested possible anticancer activity against MG-63: osteosarcoma cell line. Furthermore, our study sheds the light on the importance of Co, W and Cu as promising alloying elements. However, the antibacterial activity of the examined alloys is still questionable.
Clinical relevance: The novel nanostructured biodegradable Fe–Mn–Cu, Fe–Mn–W, and Fe–Mn–Co metal alloys offer promising bioactive materials for reconstruction of bony defects following traumatic fractures or surgical excision of tumors, In addition, they could be excellent alternatives for undegradable or non-resorbable alloys that are commonly used. Moreover, they could be used as beneficial 3D printing materials to obtain patient-specific medical implants that favor bone regeneration in addition to manufacturing of plates and screws suitable for fracture fixation.

1. Introduction

The function and aesthetic values of oral and maxillofacial regions have a significant impact on patients' well-being and quality of life. This gave prime importance to any proposed advances in the materials used for reconstruction of this area [1]. Stainless steel as well as titanium and cobalt chromium alloys have long been used as suitable bone implants. Despite their high corrosion resistance, good biocompatibility and excellent mechanical properties, being unable to degrade themselves and remain in the human body for long time has resulted in many complications. Consequently, this raises the need for a secondary removal surgery which in turn will cause health and economic burdens [2–4].

Development of nanostructured biodegradable alloys has generated a great deal of interest in the recent years owing to their unique characteristics of the nanoparticles used in term of increasing their surface area with subsequent increase in their surface reactivity, dominance of electromagnetic forces of these nanoparticles as well as their random molecular motion. These novel alloys offer promising bioactive materials for reconstruction of bony defects following traumatic fractures or surgical excision of tumors, that can conquer the drawbacks of the commonly used metallic materials. Owing to the various advantages of biodegradable metals, their medical applications have been extensively studied in the past decade [5]. Among them, iron (Fe)-based alloys were investigated and showed high strength, good biocompatibility and mechanical properties [6]. Nevertheless, its degradation rate is too low, it releases insoluble products and hinders the use of magnetic resonance imaging. Fortunately, alloying with manganese (Mn) overcame these limitations, where it increased degradation rate, improved the mechanical properties, strength, and formability in addition to enabling magnetic resonance imaging [7–9].

Recently, increasing research interests are shifting toward enhancing the properties of biodegradable metal alloys by adding more metals using advances of nanotechnology. Among these metals, copper (Cu), tungsten (W), and cobalt (Co) were added to Fe–Mn-based biodegradable alloys [10]. Cu is a critical component that has many biological activities and benefits, which renders it a promising element for development of new Cu-containing biomaterials with improved healing mechanisms [11]. On the other hand, W is well known for its inertness and stability, that's why it has long been an attractive candidate in several medical fields. Moreover, it has unique characteristics as hardness, ability to resist buckling forces at small dimensions as well as high tensile strength [12].

Furthermore, cobalt based alloys have been used for biomedical implants for several years, where they exhibited good mechanical properties [13]. Accordingly, it seemed very interesting to investigate the biocompatibility of novel nanostructured Fe–Mn-based biodegradable (Fe–Mn–Cu, Fe–Mn–W, and Fe–Mn–Co) alloys on normal oral epithelial cell line as well as their antibacterial effects against *Escherichia coli (E. coli)*. Moreover, the possible anticancer effect of such alloys was also examined on osteosarcoma cell line.

2. Materials and methods

2.1. Biodegradable alloys

Three nanostructured Fe–Mn-based biodegradable (Fe–Mn–Cu, Fe–Mn–W, and Fe–Mn–Co) alloys were used. These alloys were successfully synthesized by Ammar et al. [10] using mechanical alloying. They used high purity (99.9 %) Fe, Mn, Cu, W, and Co elemental powders with an average particle size less than 44 μm. Moreover, they tested the physical properties and confirmed that the obtained nanostructured alloys were homogenous with an even elemental dispersion of alloys. The designations and composition in atomic percentage of the 4 examined nanostructured alloys were listed in the following table [1].

2.2. Cell cultures

Two cell lines were purchased from Nawah Scientific Inc (Al-Mokattam, Cairo, Egypt), MG-63: osteosarcoma and oral epithelial OEC. Cells were maintained in DMEM media supplemented with 100 mg/ml of streptomycin, 100 units/mL of penicillin and 10 % of heat-inactivated fetal bovine serum in humidified, 5 % (v/v) CO2 atmosphere at 37 °C.

2.2.1. Cytotoxicity assays

Cell viability was assessed by the sulforhodamine B (SRB) assay. Aliquots of 100 μL cell suspension (5 × 10ˆ3 cells) were incubated in complete media in 96-well plate. The aliquots were exposed to the biodegradable discs for 24 and 72 h, however unexposed aliquots were used as control (C). After exposure, cells were fixed by replacing media with 150 μL of 10 % TCA and incubated at 4 °C for 1 h. Then, TCA solution was removed, and the cells were washed 5 times with distilled water. SRB solution (70 μL) (0.4 % w/v) were added and incubated in a dark place at room temperature for 10 min, then, the plates were washed 3 times with 1 % acetic acid and allowed to air-dry overnight. Finally, 150 μL of TRIS (10 mM) was added to dissolve protein-bound SRB stain; the absorbance was measured at 540 nm using a BMG LABTECH®- FLUOstar Omega microplate reader (Ortenberg, Germany). The cultured cells were examined under the inverted phase contrast microscope at 10× magnification with scale range 50–100 μm to assure viability and adequacy of cultured cells as well as their morphology.

2.3. Antibacterial effect

The antibacterial effect of the tested materials was evaluated using disc diffusion susceptibility (Kirby-Bauer method) and colony suspension method. For preparation of the inoculum, a disc of *E. coli* ATCC 8739 was inoculated into 100 ml of Tryptic Soy Broth medium and incubated at 37 °C for 24 h. A fresh culture agar plate was prepared where a loopful from broth was streaked onto Tryptic Soy Agar (TSA) medium, incubated at 37 °C for 21 h. A direct sterile saline solution was prepared by inoculating 3–4 colonies of *E. coli*, the suspension was adjusted to achieve a turbidity equivalent to a 0.5 McFarland standard. Inoculum density was standardized by using a 0.5 McFarland standard and DensiCHEK© optical device. That adjustment resulted in a suspension containing approximately 1 × 10^8 CFU/mL of *E. coli*.

2.3.1. Disc diffusion susceptibility (Kirby-Bauer method)

Then, around 20 ml of TSA was poured at 90 mm Petri dish then the plate was left to dry. A sterile swab was dipped into the inoculum tube. The dried surface of TSA plate was inoculated by streaking the swab 3 times over the entire agar surface, the plate was rotated approximately 60° each time to ensure an even distribution of the inoculum. The provided disk was placed on the surface of the agar using sterile forceps. Finally, the plate was incubated at 37 for 24 h.

2.4. Colony suspension method

The adjusted suspension was diluted to 1 × 107 CFU/ml by taking 1 ml and inoculates it in 9 ml buffered peptone water (BPW). This step was repeated to obtain dilution of 1 × 106 CFU/ml. Each disc was immersed in 10 ml BPW and stored at 37 °C. After 4 h, 1 ml from the solution was aspirated and inoculated in single well. 1 ml of the prepared inoculum was added to each well, resulting in a concentration of 5 × 105 CFU/ml. Same Steps were followed for 24 h and 72 h at 37 °C, respectively. Moreover, a growth control well containing inoculated broth, without sample, as well as a negative control well containing only the broth without sample or bacteria was added to each sample plate. All Plates were incubated at 37 °C for 24 h. Finally, the wells were checked for any visual growth and optical density of the inoculated wells was measured at 600 nm.

2.5. Statistical analysis

The data obtained from three independent series of experiments were used to calculate mean values ± SD. The variance analysis (ANOVA) and Tukey's HSD test were performed using the statistical package for the social sciences (SPSS) version 26. The p-value <0.05 was considered significant.

3. Results

3.1. Cytotoxicity and cell viability

In OEC cell line, the cell viability percentage showed significant increase in all the experimental groups (M1, M2, M3) in comparison to the control group. The highest percentage was observed in M3 group, followed by M2 then M1, at 24 and 72-h intervals, respectively. Multiple pairwise comparisons revealed significant differences on comparing each 2 groups together at both 24 and 72-h intervals (Tables 1 and 2) (Fig. 1a and 2).

In OS cell line, the cell viability percentage showed significant increase in all the experimental groups (M1, M2, M3) in comparison to the control group, however multiple pairwise comparisons revealed insignificant difference among M1, M2 and M3 groups at 24-h

Table (1)
The designations and composition in atomic percentage of the 3 examined nanostructured alloys.

Experimental group	Nanostructured Alloy	Atomic percentage
M1	Fe-Mn-CU	All have the same anatomical percentage 65:32:3
M2	Fe–Mn–W	
M3	Fe–Mn–Co	

Table (2)
Mean values of cell viability percentage in the experimental groups and the *P*-values among them at 24 and 72-h intervals.

Cell Line		C	M1	M2	M3	*P*-value
OEC	24 h	5.087 ± 0.103^{a}	28.314 ± 1.350^{b}	31.440 ± 0.868^{c}	34.015 ± 0.764^{d}	0.000
	72 h	5.296 ± 0.071^{a}	29.559 ± 1.463^{b}	33.161 ± 1.386^{c}	37.373 ± 1.598^{d}	0.000
OS	24 h	4.281 ± 0.140^{a}	7.698 ± 1.563^{b}	7.178 ± 0.482^{b}	7.700 ± 0.538^{b}	0.0034
	72 h	4.486 ± 0.294^{a}	0.505 ± 0.277^{b}	1.186 ± 0.064^{b}	2.311 ± 0.381^{c}	0.000

*Subscripts with different letters within the same row are statistically significant, while Subscripts with the same letters are statistically non-significant.

interval. On the contrary, at 72-h interval, the cell viability percentage showed significant drop in all the study groups (M1, M2, M3) in comparison to the control group. The lowest percentage was observed in M1 group, followed by M2 then M3. Multiple pairwise comparisons revealed significant differences between each 2 groups together at 72-h interval except for groups M1 versus M2 which showed insignificant difference (Tables 1 and 2) (Fig. 1b and Fig. 3).

3.2. Antibacterial effect

All the examined study groups didn't show any evidence of anti-bacterial effect against E. coli reference culture, where no inhibition zones were detected at 24 and 72-h interval [Fig. 4 (A,B)].

4. Discussion

Fe-based biodegradable alloys as next-generation bone implants has attracted considerable attention in the last decade, this is attributed to their outstanding mechanical properties and favorable biocompatibility [4]. To extend freedom in alloy design and to upgrade the mechanical properties and degradation rates of Fe–Mn alloys, novel design strategies have been presented [1]. According to this approach, Ammar et al. [10] produced Fe–Mn–Cu, Fe–Mn–W, and Fe–Mn–Co alloys, which were investigated in the current work. While alloying with Cu was previously reported and has shown evident influence on density, hardness, and biodegradation rate (about 2 times higher) of Fe [14,15], few trials reported successful addition of W and Co to Fe–Mn-based alloys [16,17].

Biocompatibility is defined as "the ability of a biomaterial to perform its desired function without eliciting any undesirable local or systemic effects on the recipient". Testing the biocompatibility of any biomaterial is a primary requirement in its approval for clinical use [18].Accordingly, in vitro cytotoxicity of the novel (Fe–Mn–Cu, Fe–Mn–W, and Fe–Mn–Co) alloys was assessed on cultured cells as described by the International Standards Organization (ISO 10993-5) [19]. In the current study, all of the examined alloys exhibited good biocompatibility with clearly enhanced cell proliferation in a time dependent manner. In agreement with our results, several researches revealed evident biocompatibility of Fe–Mn-based biodegradable alloys [8,20,21]. In the same line, the ability of Fe to induce proliferation of epithelial cells, deposition of extracellular proteins and remodeling of extracellular matrix has been reported in previous studies [22,23]. The optimum biocompatibility was reached by the alloy with Co content, this may be attributed to the ability of low doses of Co to mimic hypoxia and upregulate hypoxia inducible factor-1α in many cell types, which enhanced cell responses [24,25].

It's worth noting that the antibacterial activity of metal alloys is crucial to eradicate the possibility of implant-related infections which seriously cause implantation failure and threaten human health [26]. Several studies confirmed the antibacterial effect of

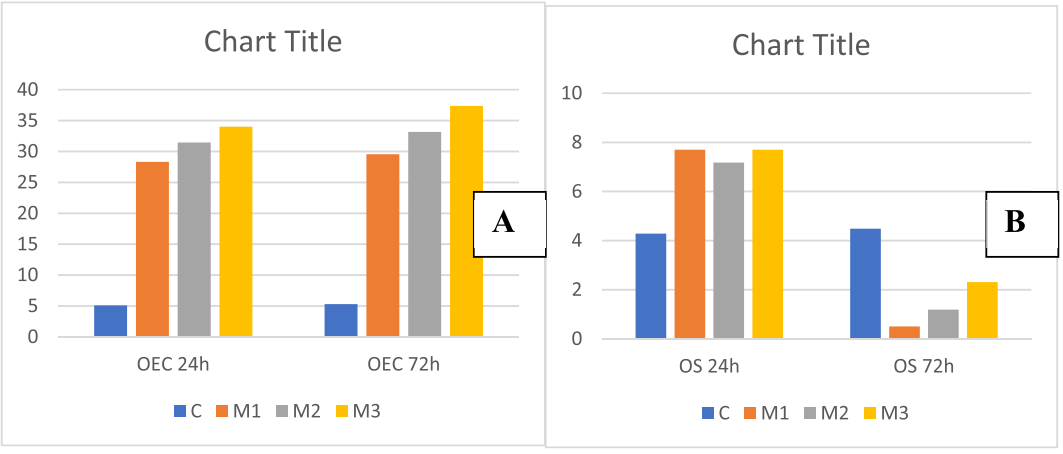

Fig (1). Column charts showing the mean values of cell viability percentage in normal oral epithelial cell line (A) and osteosarcoma cell line (B) at 24 and 72-h intervals.

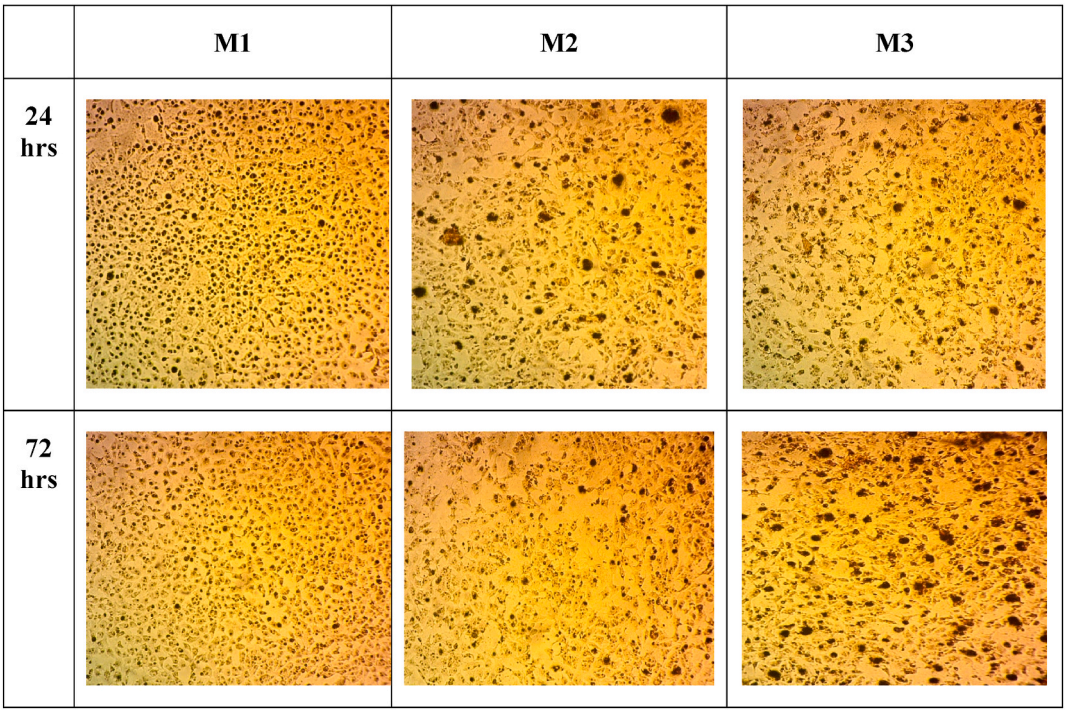

Fig (2). ***OEC cell line*** with Inverted phase microscopic pictures at 10× magnification with scale range 50–100 μm showing the morphology of viable epithelial cells at 24 and 72 h time intervals. Most of the cells are polyhedral and maintain inter-cellular adhesion with the adjacent cells.

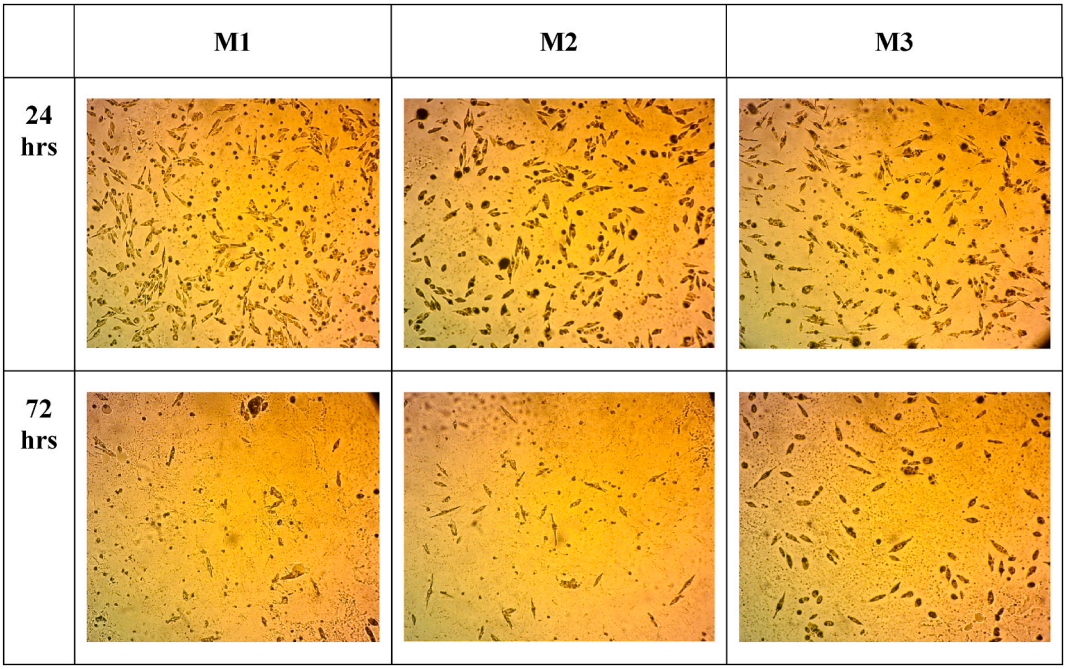

Fig (3). ***OS cell line*** with Inverted phase microscopic pictures at 10× magnification with scale range 50–100 μm showing obvious reduction of viable osteosarcoma cells from 24 to 72 h time intervals, especially in M1 followed by M2 then M3. Most of the cells are spindle in shape.

Fe–Mn based alloys [7,27]. So far, it is widely accepted that silver (Ag) [27] and Cu [7,14,15] are the most promising alloying elements that have largely enhanced the antibacterial effect of the base alloys. Studies showed superior antibacterial activity of Ag more than Cu. However, there are specific types of bacteria having more amine and carboxylic groups on the cell surface that have more affinity

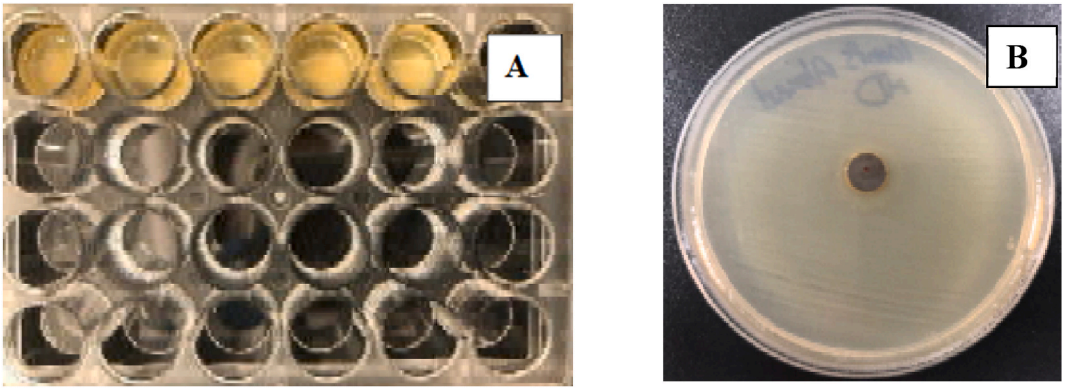

Fig (4). Photos of a 24-well plate (A) and a Petri dish (B) containing *E*-coli colonies and showing no inhibition zones.

to Cu, thus Cu can easily disrupt their nucleic acids and key enzymes. As known that Nano Cu oxide could reduce populations of Gram + ve and Gram-ve bacteria by 68 % and 65 % respectively. Moreover, while Co and Cu resisted bacterial attachment and growth effectively, W was also listed among the metals that have exhibited antibacterial effects to some extent [26].

In the current study, all the nanostructured metals, namely Cu, W, and Co that were selected and added to Fe–Mn-based biodegradable alloys, due to their well-recognized potent biological antibacterial properties [10–12,34]. Unfortunately, this study failed to prove the antibacterial efficacy of the novel nanostructures as all the examined study groups didn't show any evidence of anti-bacterial effect against E. coli reference culture, where no inhibition zones were detected at 24 and 72-h interval. This might designate the limited antibacterial potential of the combined metals to perform the required synergistic antibacterial action. As the antibacterial activity of the examined alloys is still questionable, further studies are recommended to re-evaluate the antibacterial effect of the examined alloys using different testing protocols and different species with more time intervals and to point out the possible modifications to upgrade this activity in such alloys.

To date, the available literature lacks any evidence about antitumor effect of Fe–Mn-based biodegradable alloys, this could be attributed to the recency of the research point. For the best of our knowledge, this study is one of the leading research projects to unravel the possible anticancer effect of such alloys. In the same context, the potential antitumor properties of biodegradable Mg-based alloys both in vitro and in vivo has been elucidated [28,29]. Interestingly, all the examined alloys exerted a selective time dependent cytotoxic effect on cancer cells rather than normal cell lines. This may be attributed to production of Fe^{2+} and Fe^{3+} ions, as well as reactive oxygen species (ROS) and free radicals, which may exert cytotoxic effects on the cells [30]. The most potent anticancer activity in the present study was achieved by the alloy with Cu content. Some recent studies have proposed the possible anticancer effect of Cu [31–33].

Interestingly, the significant increase in cell viability percentage of OS cell line at 24-h interval, and the subsequent drop at 72-h interval could be attributed to the hermetic effect of the produced ROS. This effect is expressed by biphasic action: stimulation by low doses and inhibition by high doses. This could clarify that the limited amount of ROS at 24-h interval has stimulated the activities of the OS cell line. Contrarily, the time dependent increase in production of ROS and their accumulation at 72-h interval, exerted a cytotoxic and inhibitory effect on OS cell line [34].The current work affords new insights on the dual biofunction of novel nanostructured biodegradable metal alloys, namely Fe–Mn–Cu, Fe–Mn–W, and Fe–Mn–Co. They showed two contradictory favorable effects biocompatibility and enhancement of cell proliferation on normal cells as well as cytotoxic activity on tumor cells. This selectivity renders the examined alloys suitable for reconstruction of bony defects following surgical excision of malignant tumors with high recurrence rate.

Owing to their good biocompatibility, they could be used as beneficial 3D printing materials to obtain patient-specific medical implants that favor bone regeneration in addition to manufacturing of plates and screws suitable for fracture fixation. In accordance with our results, most previous preclinical studies on such alloys have yielded hopeful results. However, their applications in the oral and maxillofacial regions is still taking its first steps [1]. This raises a compelling need for further investigations in these research fields to obtain novels alloys with great potential in future applications.

LIMITATION OF THE STUDY:

As any laboratory study there are several inevitable limitations that might affect the clinical consideration as the humidity, temperature, acidity and the harbor of different microbial species. However, this preclinical study is very essential to accurately determine and properly interpret the mechanism of action of this novel alloys in bone regeneration and osteointegration as well as their biocompatibility, antibacterial activity and carcinogenicity before clinical evaluation.

5. Conclusions

1. The novel nanostructured biodegradable Fe–Mn–Cu, Fe–Mn–W, and Fe–Mn–Co metal alloys exhibit good biocompatibility on oral epithelial cell line with enhancement of cell proliferation in a time dependent manner, where Fe–Mn–Co showed the highest biocompatibility.

2. On the other hand, all the alloys manifested possible anticancer activity against MG-63: osteosarcoma cell line, especially Fe–Mn–Cu.
3. Furthermore, our study sheds the light on the importance of Co, W and Cu as promising alloying elements.
4. However, the antibacterial activity of the examined alloys is still questionable.
5. The novel nanostructured biodegradable Fe–Mn–Cu, Fe–Mn–W, and Fe–Mn–Co metal alloys could be considered as excellent alternative for undegradable or non-resorbable alloys that may have some complications and subsequent need for a secondary removal surgery accompanied by risk on patient health and extra financial demands.
6. These novel alloys offer promising bioactive materials for reconstruction of bony defects following traumatic fractures or surgical excision of tumors, that can overcome the drawbacks of the commonly used metallic materials.

CRediT authorship contribution statement

Samir El Borolosy: Data curation, Formal analysis, Investigation, Methodology, Resources, Writing – original draft, Writing – review & editing, Supervision, Validation, Visualization, Conceptualization, Funding acquisition. **Lamis Ahmed Hussein:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Writing – original draft, Writing – review & editing, Funding acquisition, Software. **Hamada Mahran:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Hany R. Ammar:** Conceptualization, Data curation, Formal analysis, Investigation, Resources, Writing – review & editing. **S. Sivasankaran:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Writing – review & editing. **Safa Fathy Abd El-Ghani:** Conceptualization, Data curation, Formal analysis, Software, Writing – original draft, Writing – review & editing. **Mohamed Yehia Abdelfattah:** Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing, Resources. **Ahmed Wael Abou-Zeid:** Data curation, Formal analysis, Investigation, Software, Writing – original draft, Writing – review & editing. **Shereen Hafez Ibrahim:** Formal analysis, Writing – review & editing. **Mohamed El Shamaa:** Data curation, Formal analysis, Investigation, Methodology, Resources, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

The authors would like to acknowledge Dr. Bandar AlMangour for his help.

List of abbreviations

Fe	iron
Mn	manganese
Cu	copper
W	tungsten
Co	cobalt
E. coli	Escherichia coli
MG-63	osteosarcoma and oral epithelial OEC
OS	osteosarcoma cell line
DMEM media	Dulbecco's Modified Eagle Medium
TCA	trichloric acetic acid
SRB assay	Sulforhodamine B assay
BPW	buffered peptone water
ROS	reactive oxygen species

References

[1] D. Xia, F. Yang, Y. Zheng, Y. Liu, Y. Zhou, Research status of biodegradable metals designed for oral and maxillofacial applications: a review, Bioact Mater. 6 (11) (2021) 4186–4208, https://doi.org/10.1016/j.bioactmat.2021.01.011. PMID: 33997502; PMCID: PMC8099919.

[2] S. Thiyavimal, E. F Durán-Lara, S. Vasantharaj, M. Saravanan, A. Sabour, M. Alshiekheid, N.T. Lan Chi, K. Brindhadevi, A. Pugazhendhi, Green synthesis of copper oxide nanoparticles using Abutilon indicum leaves extract and their evaluation of antibacterial, anticancer in human A549 lung and MDA-MB-231 breast cancer cells, Food Chem. Toxicol. 168 (2022), 113330, https://doi.org/10.1016/j.fct.2022.113330. Epub 2022 Aug 1. PMID: 35926645.

[3] Jin-long Su, Jie Teng, Zi-li Xu, Li Yuan, Biodegradable magnesium-matrix composites: a review, Int. J. Miner. Metall. Mater. 27 (6) (2020) 724–744, https://doi.org/10.1007/s12613-020-1987-2.

[4] ShengLiabJianzengRenabXiangdingWangabYuejiaoDingabPengweiLiabYifanHucYouwenYang, Dilemmas and countermeasures of Fe-based biomaterials for next-generation bone implants, J. Mater. Res. Technol. 20 (2022) 2034–2205. September–October.

[5] S. Vujović, J. Desnica, D. Stanišić, I. Ognjanović, M. Stevanovic, G. Rosic, Applications of biodegradable magnesium-based materials in reconstructive oral and maxillofacial surgery: a review, Molecules 27 (17) (2022) 5529, https://doi.org/10.3390/molecules27175529. PMID: 36080296; PMCID: PMC9457564.

[6] R. Gorejová, L. Haverová, R. Oriňaková, A. Oriňak, M. Oriňak, Recent advancements in Fe-based biodegradable materials for bone repair, J. Mat. Sci. 54 (3) (2019) 1913–1947, https://doi.org/10.1007/s10853-018-3011-z.

[7] Santanu Mandala, Vijay Kishoreb, Madhuparna Bose, Samit Kumar, Nandi, Mangal Roya, In vitro and in vivo degradability, biocompatibility and antimicrobial characteristics of Cu added iron-manganese alloy, J. Mater. Sci. Technol. 84 (2021) 159–172, 10 September.

[8] J.T. Krüger, K.P. Hoyer, J. Huang, V. Filor, R.H. Mateus-Vargas, H. Oltmanns, J. Meißner, G. Grundmeier, M. Schaper, FeMn with phases of a degradable Ag alloy for residue-free and adapted bioresorbability, J. Funct. Biomater. 13 (4) (2022) 185, https://doi.org/10.3390/jfb13040185. PMID: 36278654; PMCID: PMC9590034.

[9] C. Tonna, C. Wang, D. Mei, S.V. Lamaka, M.L. Zheludkevich, J. Buhagiar, Biodegradation behavior of Fe-based alloys in Hanks' Balanced Salt Solutions: Part I. material characterisation and corrosion testing, Bioact. Mater. 7 (2022) 426–440.

[10] H.R. Ammar, S. Sivasankaran, A.S. Alaboodi, Investigation of the microstructure and compressibility of biodegradable Fe-Mn-Cu/W/Co nanostructured alloy powders synthesized by mechanical alloying, Materials 14 (11) (2021) 3088, https://doi.org/10.3390/ma14113088. PMID: 34200057; PMCID: PMC8200240.

[11] Y. Xu, Y. Xu, W. Zhang, M. Li, H.P. Wendel, J. Geis-Gerstorfer, P. Li, G. Wan, S. Xu, T. Hu, Biodegradable Zn-Cu-Fe alloy as a promising material for craniomaxillofacial implants: an in vitro investigation into degradation behavior, cytotoxicity, and hemocompatibility, Front. Chem. 10 (2022), 860040, https://doi.org/10.3389/fchem.2022.860040. PMID: 35734444; PMCID: PMC9208203.

[12] A. Shah Idil, N. Donaldson, The use of tungsten as a chronically implanted material, J. Neural. Eng. 15 (2) (2018), 021006, https://doi.org/10.1088/1741-2552/aaa502. PMID: 29300000.

[13] Aherwar Amit, Amit Kumar Singh, Amar Patnaik, Cobalt based alloy: a better choice biomaterial for hip implants, Trends Biomater. Artif. Organs 30 (1) (2016) 50–55.

[14] Z. Ma, M. Gao, D. Na, Y. Li, L. Tan, K. Yang, Study on a biodegradable antibacterial Fe-Mn-C-Cu alloy as urinary implant material, Mater Sci Eng C Mater Biol Appl 103 (2019), 109718, https://doi.org/10.1016/j.msec.2019.05.003. Epub 2019 May 3. PMID: 31349483.

[15] B. Deng, Y. Guo, M.C. Zhao, Q.F. Li, B. Ma, B. Duan, D. Yin, A. Atrens, Study on a novel biodegradable and antibacterial Fe-based alloy prepared by microwave sintering, Materials 14 (14) (2021) 3784, https://doi.org/10.3390/ma14143784. PMID: 34300703; PMCID: PMC8303899.

[16] Takeshi Nagase, Mitsuharu Todai, Takayoshi Nakano, Development of Co–Cr–Mo–Fe–Mn–W and Co–Cr–Mo–Fe–Mn–W–Ag high-entropy alloys based on Co–Cr–Mo, Alloys 61 (4) (2020) 567–576, https://doi.org/10.2320/matertrans.MT-MK2019002.

[17] Lei Wang, Reza Darvishi Kamachali, Density-based grain boundary phase diagrams: application to Fe-Mn-Cr, Fe-Mn-Ni, Fe-Mn-Co, Fe-Cr-Ni and Fe-Cr-Co alloy systems, Acta Mater. 207 (2021), 116668, https://doi.org/10.1016/j.actamat.2021.116668.

[18] B. Huzum, B. Puha, R.M. Necoara, S. Gheorghevici, G. Puha, A. Filip, P.D. Sirbu, O. Alexa, Biocompatibility assessment of biomaterials used in orthopedic devices: An overview (Review). Exp Ther Med 22 (5) (2021) 1315, https://doi.org/10.3892/etm.2021.10750. Epub 2021 Sep 17. PMID: 34630669; PMCID: PMC8461597.

[19] Biological evaluation of medical devices - Part 5: Tests for in vitro cytotoxicity, 3rd ed. 1:10993-5 https://www.iso.org/obp/ui/#iso:std:iso: ISO 10993-5:2009 (en).

[20] P. Liu, D. Zhang, Y. Dai, J. Lin, Y. Li, C. Wen, Microstructure, mechanical properties, degradation behavior, and biocompatibility of porous Fe-Mn alloys fabricated by sponge impregnation and sintering techniques, Acta Biomater. 114 (2020) 485–496, https://doi.org/10.1016/j.actbio.2020.07.048. Epub 2020 Jul 29. PMID: 32738505.

[21] V.P.M. Rabeeh, T. Hanas, Progress in manufacturing and processing of degradable Fe-based implants: a review, Prog Biomater 11 (2) (2022) 163–191, https://doi.org/10.1007/s40204-022-00189-4. Epub 2022 May 18. PMID: 35583848; PMCID: PMC9156655.

[22] H.N. Wilkinson, S.E. Upson, K.L. Banyard, R. Knight, K.A. Mace, M.J. Hardman, Reduced iron in diabetic wounds: an oxidative stress-dependent role for STEAP3 in extracellular matrix deposition and remodeling, J. Invest. Dermatol. 139 (11) (2019) 2368–2377.e7, https://doi.org/10.1016/j.jid.2019.05.014. Epub 2019 Jun 7. PMID: 31176711.

[23] S. Recalcati, E. Gammella, P. Buratti, A. Doni, A. Anselmo, M. Locati, G. Cairo, Macrophage ferroportin is essential for stromal cell proliferation in wound healing, Haematologica 104 (1) (2019) 47–58, https://doi.org/10.3324/haematol, 2018.197517. Epub 2018 Aug 16. PMID: 30115660; PMCID: PMC6312033.

[24] C. Wu, Y. Zhou, W. Fan, P. Han, J. Chang, J. Yuen, M. Zhang, Y. Xiao, Hypoxia-mimicking mesoporous bioactive glass scaffolds with controllable cobalt ion release for bone tissue engineering, Biomaterials 33 (7) (2012) 2076–2085, https://doi.org/10.1016/j.biomaterials.2011.11.042. Epub 2011 Dec 15. PMID: 22177618.

[25] T. Zan, Z. Du, H. Li, Q. Li, B. Gu, Cobalt chloride improves angiogenic potential of CD133+ cells, Front Biosci (Landmark Ed) 17 (6) (2012) 2247–2258, https://doi.org/10.2741/4048. PMID: 22652775.

[26] E. Zhang, X. Zhao, J. Hu, R. Wang, S. Fu, G. Qin, Antibacterial metals and alloys for potential biomedical implants, Bioact. Mater. 6 (8) (2021) 2569–2612, https://doi.org/10.1016/j.bioactmat.2021.01.030. PMID: 33615045; PMCID: PMC7876544.

[27] P. Sotoudehbagha, S. Sheibani, M. Khakbiz, S. Ebrahimi-Barough, H. Hermawan, Novel antibacterial biodegradable Fe-Mn-Ag alloys produced by mechanical alloying, Mater Sci Eng C Mater Biol Appl 88 (2018) 88–94, https://doi.org/10.1016/j.msec.2018.03.005. Epub 2018 Mar 11. PMID: 29636142.

[28] T. Li, W. Xu, C. Liu, J. He, Q. Wang, D. Zhang, K. Sui, Z. Zhang, H. Sun, K. Yang, L. Tan, H. Shao, Anticancer effect of biodegradable magnesium on hepatobiliary carcinoma: an in vitro and in vivo study, ACS Biomater. Sci. Eng. 7 (6) (2021) 2774–2782, https://doi.org/10.1021/acsbiomaterials.1c00288. Epub 2021 May 24. PMID: 34030441.

[29] N. Anisimova, M. Kiselevskiy, N. Martynenko, R. Willumeit-Römer, E. Kornyushenkov, M. Rodionov, S. Dobatkin, Y. Estrin, Anti-tumour activity of Mg-6%Ag and Mg-10%Gd alloys in mice with inoculated melanoma, Mater Sci Eng C Mater Biol Appl 130 (2021), 112464, https://doi.org/10.1016/j.msec.2021.112464. Epub 2021 Sep 29. PMID: 34702539.

[30] E. Scarcello, D. Lison, Are Fe-based stenting materials biocompatible? A critical review of *in vitro* and *in vivo* studies, J. Funct. Biomater. 11 (1) (2019) 2, https://doi.org/10.3390/jfb11010002. PMID: 31877701; PMCID: PMC7151573.

[31] A. Hussain, M.F. AlAjmi, M.T. Rehman, S. Amir, F.M. Husain, A. Alsalme, M.A. Siddiqui, A.A. AlKhedhairy, R.A. Khan, Copper(II) complexes as potential anticancer and Nonsteroidal anti-inflammatory agents: in vitro and in vivo studies, Sci. Rep. 9 (1) (2019) 5237, https://doi.org/10.1038/s41598-019-41063-x. PMID: 30918270; PMCID: PMC6437194.

[32] C. Molinaro, A. Martoriati, L. Pelinski, K. Cailliau, Copper complexes as anticancer agents targeting topoisomerases I and II, Cancers 12 (10) (2020) 2863, https://doi.org/10.3390/cancers12102863. PMID: 33027952; PMCID: PMC7601307.

[33] S. Sathiyavimal, E. F Durán-Lara, S. Vasantharaj, M. Saravanan, A. Sabour, M. Alshiekheid, N.T. Lan Chi, K. Brindhadevi, A. Pugazhendhi, Green synthesis of copper oxide nanoparticles using Abutilon indicum leaves extract and their evaluation of antibacterial,anticancer in human A549 lung and MDA-MB-231 breast cancer cells, Food Chem. Toxicol. 168 (2022), 113330, https://doi.org/10.1016/j.fct.2022.113330. Epub 2022 Aug 1. PMID: 35926645.

[34] G. Chkadua, E. Nozadze, L. Tsakadze, L. Shioshvili, N. Arutinova, M. Leladze, S. Dzneladze, M. Javakhishvili, Effect of H_2O_2 on Na,K-ATPase, J. Bioenerg. Biomembr. 54 (5–6) (2022) 241–249, https://doi.org/10.1007/s10863-022-09948-1. Epub 2022 Nov 4. PMID: 36331672.