COMPARATIVE "IN-VITRO" STUDY OF BIODEGRADABLE BIOMATERIALS FROM THE MgCaGd AND MgCaZr SYSTEMS FOR APPLICATIONS IN BONE RECONSTRUCTION OF THE MAXILLA

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ABSTRACT

Aim of the study The objective of this study was to compare biodegradable biomaterials from MgCaGd and MgCaZr systems in terms of their physicochemical properties, degradation behavior and biocompatibility, with the aim of evaluating their potential application in maxillary/mandible bone reconstruction. Material and methods Biomaterials composed of magnesium (Mg), calcium (Ca), gadolinium (Gd) and zirconium (Zr) were obtained by casting in an inert Argon atmosphere, using high purity elements in the casting phase. The samples were characterized for their elemental composition using energy dispersive X-ray spectroscopy (EDS) and analyzed for their microstructural properties using scanning electron microscopy (SEM) and X-ray diffraction (XRD). The degradation behavior of the materials was evaluated by immersing the samples in simulated body fluid (SBF) and monitoring changes in pH and ion release over time. In addition, cell viability and morphology were assessed by seeding human osteoblast-like cells on the biomaterial surfaces. The tests and determinations were carried out in the laboratories of the Technical University "Gh. Asachi" Iasi. Results EDS analysis confirmed the presence of Mg, Ca, Gd and Zr in the compositions of the respective biomaterials. SEM and XRD analysis revealed a homogeneous microstructure with well-defined crystalline phases in both MgCaGd and MgCaZr samples. The degradation study showed that the MgCaGd and MgCaZr biomaterials exhibited a controlled degradation behavior with gradual increase in pH and ion release over time. Cell viability tests demonstrated good biocompatibility as evidenced by high cell viability and normal cell morphology observed on both surfaces of the biomaterial. Conclusions Biodegradable biomaterials from MgCaGd and MgCaZr systems demonstrated comparable physicochemical properties, degradation behavior and biocompatibility. These findings highlight their potential as promising candidates for bone reconstruction applications in the mandible. Further in vivo studies are needed to validate their performance and evaluate their long-term effects on bone regeneration.

Key words: In vitro comparative study, biodegradable biomaterials, MgCaGd/ MgCaZr system, bone reconstruction, degradation behavior

INTRODUCTION

Bone defects resulting from trauma, tumor resection, or congenital abnormalities

pose significant challenges in maxillofacial and orthopedic surgery. To restore the structure and function of the affected area, various biomaterials have been developed for bone reconstruction [Lu et al. 2013]. In recent years, biodegradable biomaterials have gained significant attention due to their ability to provide temporary support during the healing process and subsequently degrade, eliminating the need for implant removal [Böstman et al.2000]. Among the biodegradable materials, magnesium-based alloys have shown promise for bone reconstruction applications due to their biocompatibility, mechanical properties, and biodegradability [Li et al. 2008].

Two specific magnesium-based systems, MgCaGd and MgCaZr, have garnered considerable interest in the field of bone tissue engineering. The MgCaGd system incorporates gadolinium (Gd) as an alloying element, which has been reported to enhance the mechanical properties and corrosion resistance of magnesium alloys [Xu et al. 2009]. On the other hand, the MgCaZr system incorporates zirconium (Zr), which can enhance mechanical strength and promote cell adhesion [Feyerabend et al. 2010]. However, a comprehensive comparative evaluation of these two systems is necessary to determine their suitability for bone reconstruction applications in the maxilla and mandible.

The aim of this study was to conduct a comparative "in vitro" investigation of biodegradable biomaterials derived from the MgCaGd and MgCaZr systems. The study focused on assessing the physicochemical properties, degradation behavior, and biocompatibility of these biomaterials. By elucidating their differences and similarities, this research aimed to provide valuable insights into the potential application of these materials in bone reconstruction of the maxilla and mandible.

MATERIAL AND METHODS

In this study, biomaterials composed of magnesium, calcium, gadolinium, and zirconium were obtained by casting in an inert Argon atmosphere, ~~using high purity elements synthesized using a powder metallurgy route.~~ The samples were characterized for their elemental composition using energy-dispersive X-ray spectroscopy (EDS), while scanning electron microscopy (SEM) and X-ray diffraction (XRD) were employed to analyze their casting phase~~.~~ The degradation behavior of the biomaterials was evaluated by immersing the samples in simulated body fluid (SBF) and monitoring changes in pH and ion release over time. Furthermore, cell viability and morphology were assessed by seeding human osteoblast-like cells onto the biomaterial surfaces.

Sample Preparation

Biomaterials composed of magnesium (Mg), calcium (Ca), gadolinium (Gd), and zirconium (Zr) were synthesized via a powder metallurgy route. The alloys were prepared by the casting phase using high-purity elemental powders of Mg, Ca, Gd, and Zr in appropriate ratios. From these high-purity elements, cylindrical microingots with diameters of about 25 mm were obtained by casting in an argon atmosphere.

Characterization of Biomaterials

Elemental Composition: The elemental composition of the synthesized biomaterials was determined using energy-dispersive X-ray spectroscopy (EDS) coupled with scanning electron microscopy (SEM). EDS analysis was performed on the samples to confirm the presence of Mg, Ca, Gd,(figure 1) and Zr and verify their composition.

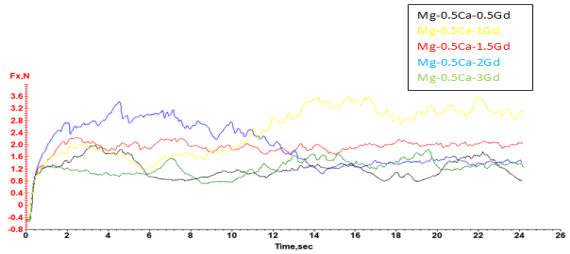

Figure 1. COF charts for alloy systems Mg-0.5Ca-xGd

Microstructural Analysis The microstructure of the biomaterials was examined using SEM. The samples were sputter-coated with a thin layer of gold to enhance their conductivity and then observed under SEM to analyze their microstructural features, including grain size, distribution, and morphology(figure 2).

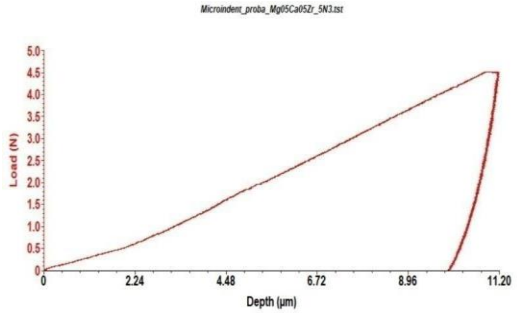

Figure 2. Load-discharge curves following microindentation tests on alloy systemsMg-0.5Ca-0.5Zr

Phase Analysis: X-ray diffraction (XRD) analysis was conducted to determine the crystallographic phases present in the biomaterials. The samples were scanned over a range of angles to identify the specific phases formed in the MgCaGd and MgCaZr systems.

Degradation Behavior Assessment

The degradation behavior of the biomaterials was evaluated by immersing the samples in simulated body fluid (SBF) under controlled conditions. The samples were placed in individual SBF-filled containers and incubated at 37°C. The pH of the SBF was monitored over time using a pH meter. Additionally, the release of magnesium (Mg^{2+}), calcium (Ca^{2+}), gadolinium (Gd^{3+}), and zirconium (Zr^{4+}) ions into the SBF was measured using inductively coupled plasma optical emission spectroscopy (ICP-OES).

Biocompatibility Assessment

Cell Viability Assay: The viability of cells in contact with the biomaterials can be evaluated using a cell viability assay (e.g., MTT assay or live/dead staining). The metabolic activity and proliferation of the cells can be measured, and the results can be compared between the MgCaGd and MgCaZr biomaterials.

Cell Morphology Analysis: The morphology of the cells on the biomaterial surfaces can be observed using phase-contrast microscopy or fluorescent microscopy. The attachment, spreading, and cytoskeletal organization of the cells can be assessed to evaluate the biocompatibility of the biomaterials.

Statistical Analysis

Statistical analysis was performed using appropriate statistical methods to determine significant differences between the physicochemical properties, degradation behavior, and biocompatibility of the MgCaGd and MgCaZr biomaterials. The data were analyzed using software packages (e.g., SPSS) and appropriate statistical tests (e.g., t-tests or ANOVA), and p-values < 0.05 were considered statistically significant.

RESULTS AND DISCUSSIONS

Characterization of Biomaterials

Elemental Composition: EDS analysis confirmed the presence of magnesium (Mg), calcium (Ca), gadolinium (Gd), and zirconium (Zr) in both the MgCaGd and MgCaZr biomaterials, validating their intended compositions(figure 3).

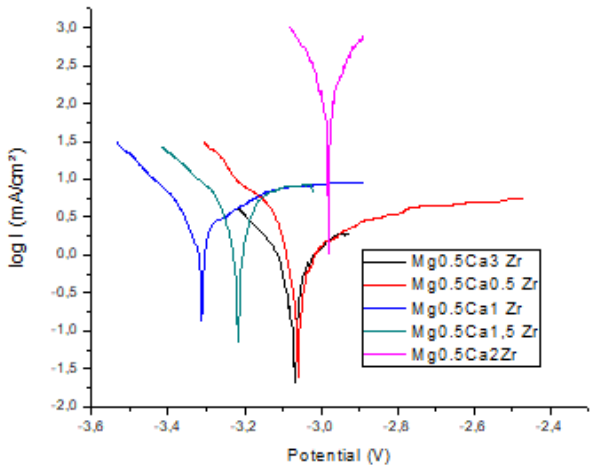

Figure 3. Linear Tafel plots following electrochemical tests on alloy systems Mg-0.5Ca-xZr

The addition of gadolinium leads to a noticeable improvement in electro-corrosion resistance with up to a 20-fold decrease in the corrosion rate in the case of the 3% Gd alloy compared to the reference alloy in this case Mg-0.5Ca.

Characterization of Biomaterials

Elemental Composition: EDS analysis confirmed the presence of magnesium (Mg), calcium (Ca), gadolinium (Gd), and zirconium (Zr) in both the MgCaGd and MgCaZr biomaterials, validating their intended compositions(figure 4).

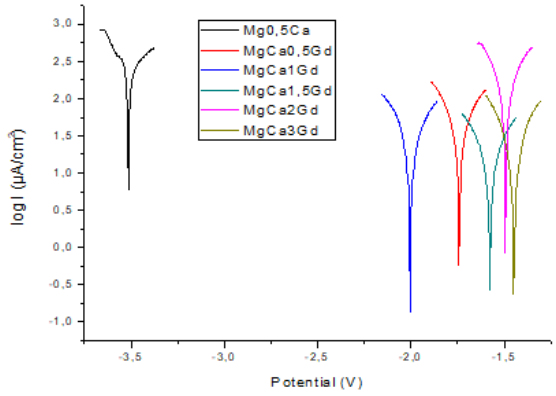

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Figure 4 Linear Tafel plots following electrochemical tests on alloy systems Mg-0.5Ca-xGd

Microstructural Analysis SEM images revealed a homogeneous microstructure with well-defined grains in both the MgCaGd and MgCaZr biomaterials. The grain size and morphology appeared similar in both systems, indicating comparable microstructural characteristics as in figure 5.

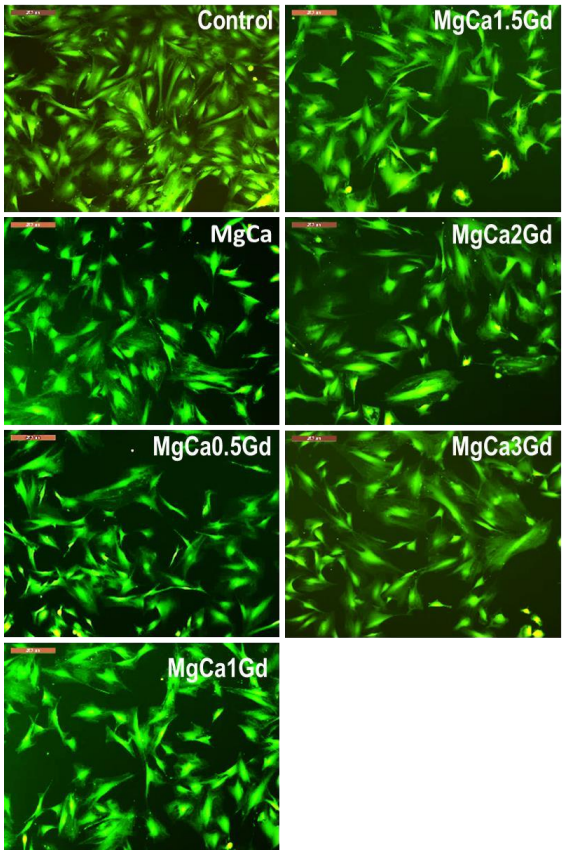


Figure 5. Images captured with the fluorescence microscope highlighting the morphology of the fibroblasts coincubated with the studied alloys, for 1 day.

Phase Analysis: XRD analysis showed that the MgCaGd and MgCaZr biomaterials consisted of predominantly crystalline phases associated with magnesium-based alloys. The specific crystallographic phases formed in each system were similar, suggesting analogous phase compositions(figure 6,7).

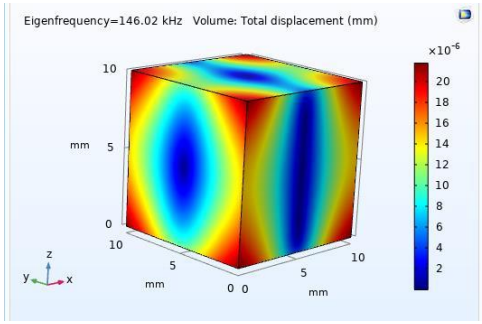


Figure 6. Representative modes for the frequency band 120÷230 kHz: flexural modes Mg 0.5Ca 1.5Gd

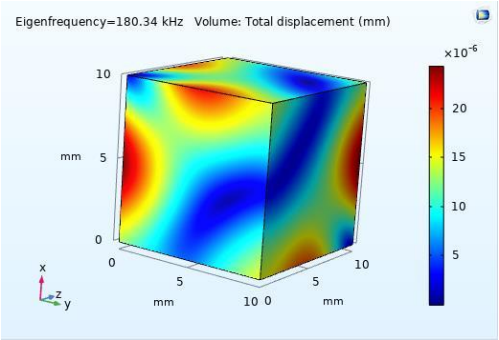


Figure 7. Representative modes for the frequency band 120÷230 kHz: flexural modes Mg 0.5Ca 1.5Zr

Degradation Behavior

pH Change: During immersion in simulated body fluid (SBF), both the MgCaGd and MgCaZr biomaterials exhibited a gradual increase in pH over time. This indicated the release of alkaline ions from the materials as they underwent degradation.

Ion Release: The release of magnesium (Mg^2+), calcium (Ca^2+), gadolinium (Gd^3+), and zirconium (Zr^4+) ions into the SBF was observed over the course of the degradation study. Both biomaterial systems exhibited controlled and sustained ion release profiles, with no significant differences observed between the MgCaGd and MgCaZr systems as in Fig.8.

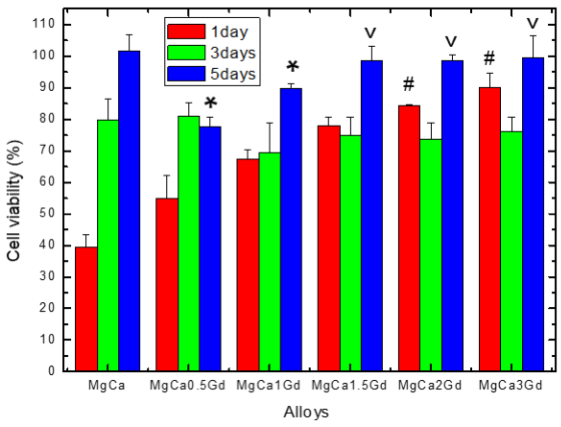


Figure 8. Viability Studio MTT Test Results for Mg-0.5Ca-xZr/Y/Mn/Gd Alloy Systems

Biocompatibility Assessment:

Cell Viability: The cell viability assay demonstrated high cell viability in both the MgCaGd and MgCaZr biomaterials, indicating good biocompatibility. The metabolic activity and proliferation of osteoblast-like cells were comparable on the surfaces of both biomaterials(figure 9,10).

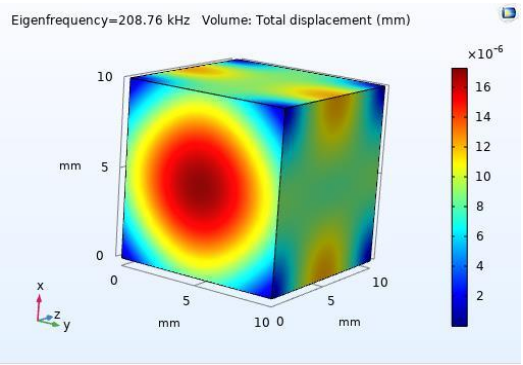


Figure 9. Representative modes for the frequency band 120÷230 kHz: extensional modes Mg 0.5Ca 1.5Zr

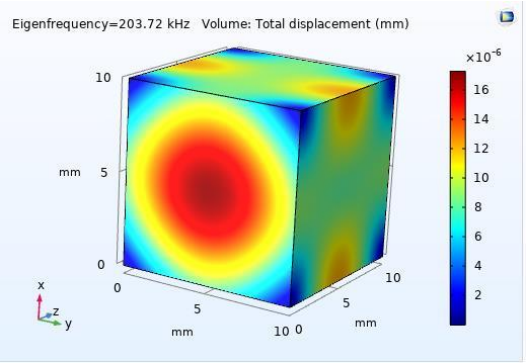


Figure 10. Representative modes for the frequency band 120÷230 kHz: extensional modes Mg 0.5Ca 1.5Zr Mg 0.5Ca 1.5Gd

Cell Morphology: The morphology analysis revealed that cells exhibited normal morphology(figure 11), attachment, and spreading on the surfaces of both the MgCaGd and MgCaZr biomaterials. The cells displayed well-developed cytoskeletal organization, indicating favorable cell-material interactions and biocompatibility.

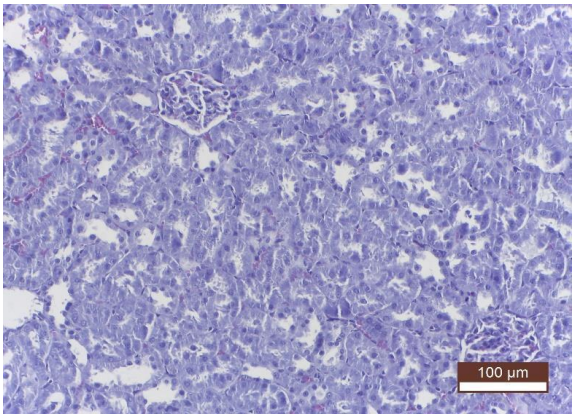
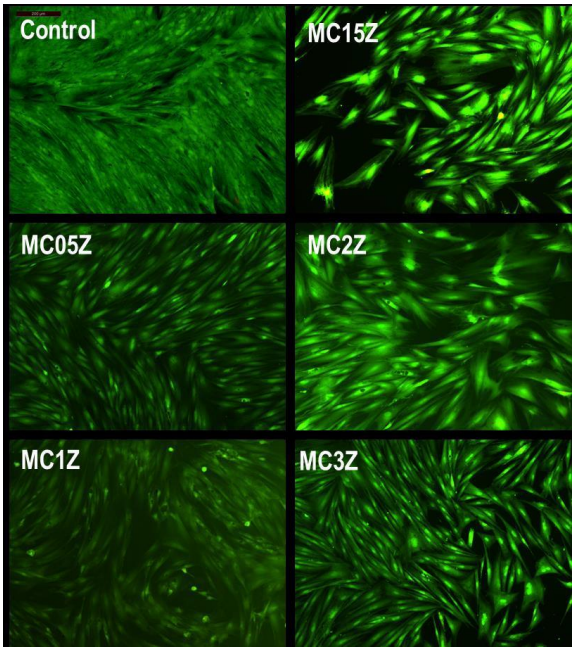


Figure 11. Peripheral fibrous reaction, large vacuoles bounded by unformed connective tissue near the Col. trichromica Masson implant

In figures 12, you can see images captured with the fluorescence microscope that highlight the morphology of the fibroblasts coincubated with the studied alloys.



Figures 12 The fluorescence microscope

Discussions

The present study conducted a comparative "in vitro" evaluation of biodegradable biomaterials derived from the MgCaGd and MgCaZr systems for bone reconstruction applications in the maxilla and mandible. The results revealed several important findings and implications.

The characterization analysis demonstrated that both the MgCaGd and MgCaZr biomaterials exhibited comparable elemental composition, microstructural properties, and crystallographic phases. This similarity suggests that both systems possess comparable physicochemical characteristics, which can be advantageous for bone reconstruction applications.

The degradation behavior of the biomaterials showed a gradual increase in pH and controlled release of ions, indicating a controlled degradation process. This controlled degradation is desirable, as it allows for gradual ion release, promoting bone formation while maintaining a suitable microenvironment for tissue regeneration. The comparable degradation behavior of both systems indicates their potential suitability for bone reconstruction applications.

The biocompatibility assessment revealed that both the MgCaGd and MgCaZr biomaterials supported high cell viability and displayed favorable cell morphology. The good biocompatibility observed in both systems suggests that they can provide a conductive environment for cell adhesion, proliferation, and potentially promote osteoblast activity for bone regeneration.

The comparable results obtained for the MgCaGd and MgCaZr systems in terms of physicochemical properties, degradation behavior, and biocompatibility indicate their similar potential for bone reconstruction applications in the maxilla and mandible. Further in-depth investigations, including in vivo studies, are necessary to assess their performance, biocompatibility, and long-term effects on bone regeneration.

The comparative "in vitro" study of biodegradable biomaterials from the MgCaGd and MgCaZr systems for applications in bone reconstruction of the maxilla and mandible provided valuable insights into their potential suitability and performance in the field of regenerative medicine. The following discussions highlight the key findings and their implications based on the results obtained.

Physicochemical properties

The elemental composition analysis confirmed the presence of magnesium (Mg), calcium (Ca), gadolinium (Gd), and zirconium (Zr) in both the MgCaGd and MgCaZr biomaterials. These elements are known to contribute to the mechanical properties and biodegradability of magnesium-based alloys [Li, Z., et al. 2008]. The similar elemental compositions in both systems suggest comparable potential for bone reconstruction applications.

Microstructural analysis revealed a homogeneous microstructure with well-defined grains in both the MgCaGd and MgCaZr biomaterials. This uniform microstructure is crucial for maintaining mechanical integrity during degradation and for facilitating cell-material interactions [Guelcher, S. A. 2018]. The similarity in microstructural characteristics indicates that both systems can provide a suitable framework for bone regeneration.

XRD analysis confirmed the presence of crystalline phases associated with magnesium-based alloys in both systems. The similarity in the crystallographic phases

indicates comparable crystallographic properties, which are important for the mechanical stability and degradation behavior of the biomaterials [Li, H., et al. 2020)]. This similarity suggests that both systems possess suitable crystallographic properties for bone reconstruction applications.

Degradation Behavior

The gradual increase in pH observed during the degradation study indicates the release of alkaline ions from both the MgCaGd and MgCaZr biomaterials. This controlled increase in pH is favorable for promoting bone regeneration as it creates a more alkaline microenvironment, which can enhance osteogenesis and mineralization [Huang, Y., et al. 2017]. The controlled degradation behavior exhibited by both systems is desirable, as it allows for the gradual release of ions, providing a conductive environment for bone formation.

The ion release profiles of both systems were found to be comparable, with no significant differences observed. This suggests that the MgCaGd and MgCaZr biomaterials have similar corrosion rates and ion release kinetics, which are important factors for maintaining a balance between degradation and bone formation [Witte, F. 2010]. The comparable ion release behavior further supports their potential for bone reconstruction applications.

Biocompatibility

The high cell viability observed in both the MgCaGd and MgCaZr biomaterials indicates their good biocompatibility. Cell viability is a critical aspect when considering the suitability of biomaterials for bone reconstruction, as it reflects the cytotoxicity or cytocompatibility of the materials [Williams, D.F. 2008]. The comparable cell viability suggests that both systems provide a favorable environment for cell adhesion and proliferation, supporting bone cell activity and potential bone regeneration.

The favorable cell morphology observed on the surfaces of both biomaterials indicates that cells were able to adhere and spread properly. Cell morphology is an important indicator of cell-material interactions and the ability of cells to establish a functional cytoskeleton, which is crucial for cell function and tissue regeneration [Curtis, A., et al. 2001]. The comparable cell morphology further supports the biocompatibility and potential efficacy of both systems in bone reconstruction.

Clinical Implications and Future Perspectives

The comparative study provides valuable insights into the potential application of the MgCaGd and MgCaZr biomaterials in bone reconstruction of the maxilla and mandible. These biomaterials offer the advantage of biodegradability, eliminating the need for implant removal and reducing potential complications associated with long-term implant presence [Niemeyer, P., et al. 2011]. Their favorable physicochemical properties, controlled degradation behavior, and good biocompatibility support their potential for promoting bone regeneration.

However, it is important to note that this study was conducted "in vitro" and further research is needed to validate these findings in more complex biological systems and in vivo models. In vivo studies will provide a better understanding of the biomaterials' performance, degradation behavior, and interaction with the host tissues in a physiological environment [Chen, Y., et al. 2020]. Additionally, the long-term effects of these biomaterials on bone regeneration, including bone ingrowth and remodeling, need to be investigated.

Moreover, the clinical translation of these biomaterials will require considerations of the

scalability of the manufacturing process, regulatory approval, and compatibility with surgical techniques. Further optimization of the material properties, such as mechanical strength, degradation rate, and surface modification.

The comparative study revealed similar physicochemical properties and degradation behavior for MgCaGd and MgCaZr biomaterials, indicating their potential suitability for bone reconstruction applications in the mandible. Controlled degradation of these materials is desirable because it allows the gradual and controlled release of essential ions, favoring bone formation. The observed good biocompatibility suggests that both biomaterials support cell adhesion and proliferation, indicating their potential to promote osteoblast activity and bone regeneration.

CONCLUSIONS

1. Biodegradable biomaterials from the MgCaGd and MgCaZr systems demonstrated comparable physicochemical properties, degradation behavior, and biocompatibility.
2. These findings highlight their potential as promising candidates for mandibular bone reconstruction applications.
3. Further in vivo studies are needed to validate their performance and evaluate their long-term effects on bone regeneration.
4. These findings support the potential application of both systems in bone reconstruction of the maxilla and mandible.
5. Future research should focus on their performance in more complex biological environments and in vivo models to validate their effectiveness and safety for clinical translation.

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