Journal of Techniques, ISSN: 2708-8383, Vol. 6, No. 3, 2024, Pages 41-45
DOI: https://doi.org/10.51173/jt.v6i3.2567

JOURNAL OF TECHNIQUES

Journal homepage*: http://journal.mtu.edu.iq*

RESEARCH ARTICLE - MATERIAL SCIENCE (MISCELLANEOUS)

Release of Copper / Magnesium Ions from Hydroxyapatite-Based Fiber Scaffolds Hasten Bone Healing and Regeneration

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Article Info. **Abstract**

Article history:

Received
12 May 2024

Accepted
17 July 2024

Publishing
30 September 2024

Releasing inorganic ions from Ca/P biomaterials could provide an alternative approach to using growth factors to improve bone healing. Two types of hydroxyapatite (HA) fiber scaffolds, copper-doped (CuHA) and magnesium-doped (MgHA), were fabricated using the sol-gel method. Two types of scaffolds were immersed in simulated body fluid (SBF), i.e., Phosphate Buffered Saline (PBS), for 30 days. The results after immersion indicated the amount of Cu and Mg ions released from the scaffolds. A low amount of Mg and Cu ions could improve vascular endothelial growth factor (VEGF) and angiogenesis around bone, thus can hasten bone healing and regeneration.

Publisher: Middle Technical University

Keywords: Scaffolds; Hydroxyapatite; Ions; Copper; Magnesium.

1. Introduction

The structure, chemistry, and mechanical properties of natural bone have been reviewed in numerous articles [1, 2]. Biological apatite deviates from the stoichiometric composition of HA and contains significant amounts of ion substitution impurities such as Na^+, Mg^{2+}, K^+, citrate, HPO^{2-}_{4}, carbonate ($CO^{2-}{}_{3}$), Cl^-, F^-, etc. In addition to the hierarchical micro/nano-structure of bone, the mineral in human bone is not pure stoichiometric HA, which is partially substituted by elements such as Na, K, Sr, Cu, and F [3]. The replaced elements not only affect the physical structure of apatite but also strengthen its biological function, such as the importance of copper ions to endothelial cells [4]. Therefore, mimicking bone structure is an excellent choice for scaffold design.

The bioactivity of the hydroxyapatite could enhanced by the inclusion of ionic substitutions, including Na^{2+}, Mg^{2+}, Sr^{2+}, Zn^{2+}, Fe3+, F, SiO_4^{4-} and CO_2^{3-}[5]. The substitution can affect the biological and mechanical properties. An ion covalent structural model of the apatite family shows a structure that accepts cationic and anionic substituents. These substitutions induce modifications in the lattice parameters and in crystallinity, which substantially influence the solubility of HA under physiological conditions without generating significant changes in the hexagonal apatite system [4]. These substitutions alter the crystal structure, inducing some extent of the structural disorder and so also changing the material's properties, including its crystallinity, stability, solubility, reactivity, crystal morphology, surface characteristics, bioactivity, and biocompatibility. There are several methods available to synthesize HA materials, which include combustion methods [6], hydrothermal methods, mechano-chemical synthesis [7], electrochemical deposition sol-gel techniques [8] and chemical precipitations [9].

Copper is well known to have a significant role in the human body, affecting blood vessel formation [10], and facilitating the release of vascular endothelial growth factor (VEGF), and cytokines from producing cells [11]. Copper, as a trace element in the human body, has a close association and role in angiogenic processes and is known to initiate endothelial cell migration toward angiogenesis [12]. Also, copper ions

Nomenclature & Symbols			
PBS	Phosphate Buffered Saline	VEGF	Vascular Endothelial Growth Factor
Mg–CS	Magnesium–Calcium Silicate Cement	CuHA	Copper doped-HA
MgHA	Magnesium Doped-HA	SEM	Scanning Electron Microscope

Substituting HA materials with Cu can improve the HA composition and biological properties [13]. A significant amount of information has been gained from understanding its role in angiogenic processes associated with tumor development [14].

Magnesium is the tenth most abundant element in the body; after potassium, it is the next most abundant. Half of the total amount of magnesium present in the body is associated with bone and appears to have two locations: one is an integral part of the HA lattice in the inorganic bone matrix, and the second is a more easily exchangeable cell surface-bound location.

Previous studies developed a fast-setting and controllably degrading magnesium–calcium silicate cement (Mg–CS) by sol-gel, which has been examined for angiogenesis during new bone formation in both in vivo and in vitro experiments with calcium silicate-based materials [15].

Among the available literature about the analysis of ions released from HA fiber scaffolds, an understanding of the effect of Cu^{2+} and Mg^{2+} ions on the biological environment is still lacking.

However, these works did not consider if Cu^{2+} or Mg^{2+} could be applied to regulate the surface morphology of HA fiber scaffolds. However, they investigated the release of Cu and Mg ions from HA fiber scaffolds. The results showed a low amount of release of both Cu and Mg ions from the scaffolds..

2. Methodology

2.1. Starting materials and synthesis methodology

All the chemical materials used in the synthesis were purchased from Kelong Chemical (Chengdu, Sichuan, China). Calcium nitrate Ca $(NO_3)_2$; diammonium phosphate $(NH_4)_2HPO_4$, DAP); magnesium nitrate (Mg $(NO_3)_2$, and Copper nitrate (Cu $(NO_3)_2$ and disodium phosphate (Na_2PO_4, DSP).

2.1.1. Synthesis of HA Nano-powder

The HA powder was synthesized by the precipitation method [16], as follows: 0.1 mol/L of Ca $(NO_3)_2{\cdot}4H_2O$ dissolved in a beaker compromised 100 mL of distilled water and formulating a range of $Ca(NO_3)_2{\cdot}4H_2O$ mixed solution and kept at (Ca/Ca = 1% and Ca/ P =1.67) constant total molar concentration, as shown in equation 1,[17]. Then, 0.06mol/L Na_2PO_4 was added drop wisely to the 30 mL of solution at a rate of 2 ml/min under string. After observing the gel formation, this gel was cooled at room temperature, centrifuged at 3500 rpm for 3 mins, and washed with distilled water further, and the product was dried in an oven at 80 ℃ for 2 hrs.

$$Ca(NO_3)_2 + 6Na_2HPO_4 + 2H_2O \rightarrow Ca_2 + (PO_4)_6 + (OH)_2 + 12NaNO_3 + 8HNO_3 \tag{1}$$

2.1.2. Synthesis of Cu doped HA and Mg doped-HA

Copper-doped HA (5CuHA) and magnesium-doped-HA (5MgHA) were synthesized using an identical precipitation method. Solution(1): contains 0.005 mol/L of $Cu(NO_3)_2$-$6H_2O$ and 0.095 mol/L of $Ca(NO_3)_2{\cdot}4H_2O$ dissolved in 100 ml of distilled water. Solution (2): compromised $Mg(NO_3)_2$-$6H_2O$ and $Ca(NO_3)_2{\cdot}4H_2O$ dissolved in 100 ml of distilled water. Then, 0.06mol/L Na_2PO_4 was added drop wisely to the 30 mL of solution at a rate of 2 ml/min under string.

The molar ratio on the initial solution was adjusted and kept at ((M + Ca)/P=1.67) and M/(M + Ca) = 5%), *i.e.*, M = Cu, Mg. Then, the solution was adjusted to the pH at value =7, as shown in Table 1. After observing the gel formation, this gel was cooled at room temperature, centrifuged at 3500 rpm for 3 min, and washed with distilled water further. The product was dried with an oven at 80 ℃ for 2 h., and later it was grinded. The equation 2 illustrated the ions substituted-HA biomaterial into the Ca^{2+} site [18]:

$$(10-x)Ca^{2+} + xM^{2+} + 6PO_4^{3-} + 2H2O \rightarrow Ca_{(10-X)}Mx(PO_4)_6(OH)_2 + 2H^+ \tag{2}$$

$$M^{2+} = Cu^{2+}, Mg^{2+} \;;\quad x = 0.5$$

Table 1. Summary of compositions of copper and magnesium ions

Sample	Composition in solutions (wt.%)				Ratio
	Cu	Mg	Ca	P	Ca/P
CuHA	0.31	0.00	37.87	18.40	1.67
MgHA	0.00	0.12	39.70	18.40	1.67

2.2. Fabrication of HA and HA-doped scaffolds

The sol-gel method was used to fabricate a series of groups of MgHA and CuHA scaffolds. Amount of 3 wt.% alginates was dissolved into 100 mL of distilled water at a ratio of 7 to 1 (HA/alginate solution, w/w). Then, separately in two beakers, we add 20 g of doped HA powders to the homogenous to synthesis HA slurry. The mixtures were steered for 12 hrs to hemogenic composite suspensions. Subsequently, the suspension composite was placed in a syringe and dispensed in a $CaCl_2$ solution (200 mM) to rapidly solidify the deposit, as shown in Fig. 1. Later, the scaffold fibrous were placed in a cylindrical mold (a syringe) and pressed down to produce cylinder-shaped scaffolds [19] (Φ 0.5×1 cm). Finally, to fabricate reinforced HA scaffolds in favor of biological implantation, the fibrous scaffolds were sintered at 1200 °C for 12 hrs, [19].

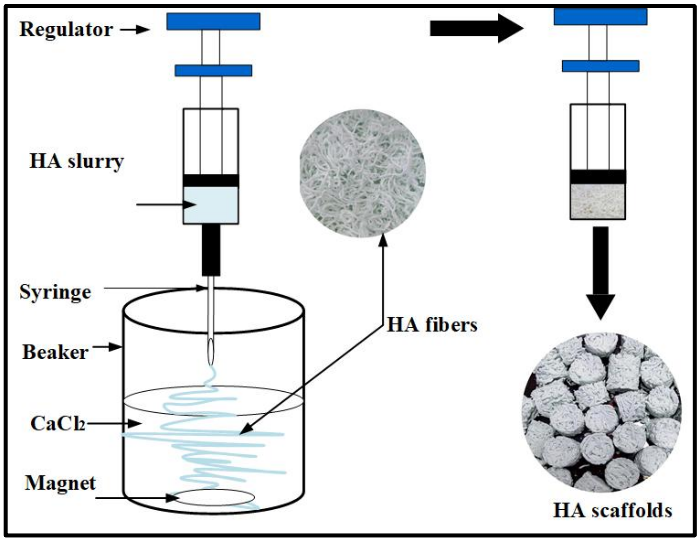

Fig. 1. The preparation of HA Fiber scaffolds by sol-gel method

3. Release Ions Assay of Cu/Mg from Fiber Scaffolds

To evaluate the ion release from CuHA and MgHA scaffolds, 0.5 grams of scaffolds were immersed in 10 μL of phosphate-buffered saline (PBS) and incubated at 37ºC in a humid atmosphere with 5% CO_2 for 1, 2, 3, and 4 weeks [20]. Three samples of CuHA and MgHA were used per material (n = 3). The scaffold particles were collected to analyze and evaluate the release of copper and magnesium ions from the HA fiber scaffolds. Both copper and magnesium concentrations in the medium were measured with a Sigma Aldrich copper assay kit (Cu, Mg, colorimetric method, China) and compared to the control (i.e., Benjamin Moore ^{R} color samples (BM samples)) flowing the manufacturer's guidelines. Absorbance measurements were performed with a spectrophotometer at both 580 & 800 nm.

4. Results and Discussion

4.1. Optical microscopic analysis characterized MgHA and CuHA scaffolds

Fig. demonstrates the optical microscopic images of MgHA and CuHA scaffolds. Fig. 2(a) illustrates as-sintered MgHA scaffolds in white color (original color), while in Fig. 2 (b), the CuHA scaffold color tends to be (chocolate-like). This phenomenon (color change) may refer to the presence of Cu-doped HA powder [20].

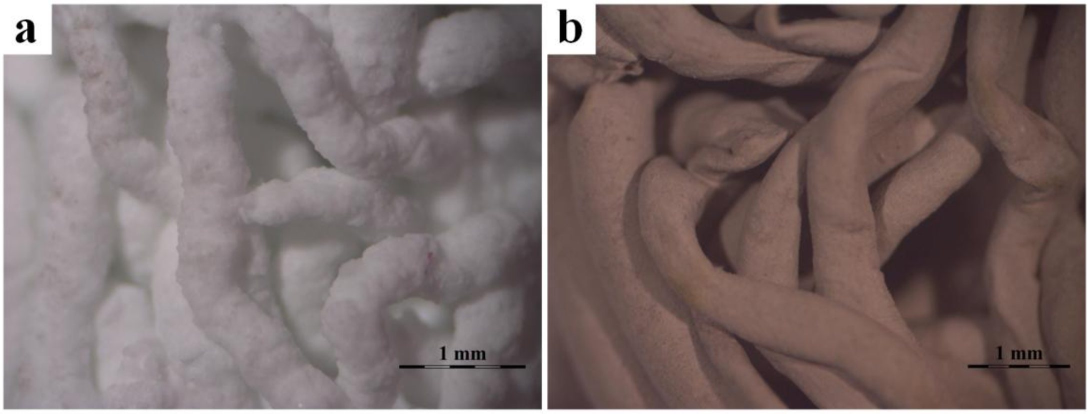

Fig. 2. SEM images; (a) MgHA, and (b) CuHA scaffolds

4.2. The release ions from the CuHA and MgHA scaffolds

As mentioned previously, the release rate of copper ions from two types of scaffolds MgHA & CuHA can be determined by the dissolution rate of the calcium phosphates and the content of substituted ions [21].

On the one hand, as shown in Fig. 3, during the period immersed in PBS, the results obtained show that a low amount of Cu and Mg ions are accumulated and released from fiber scaffolds (i.e., 15% and 20%, respectively). Thus, this may be due to the high sinter temperature of scaffolds (i,e,. 1200 °C), which is above of evaporate temperature of Mg and Cu. So, the amount of ions may earlier evaporated during sintering from the fiber scaffolds. On the other hand, according to the previous result, both Cu and Mg had a significant effect on the scaffold's degradation and conversion.

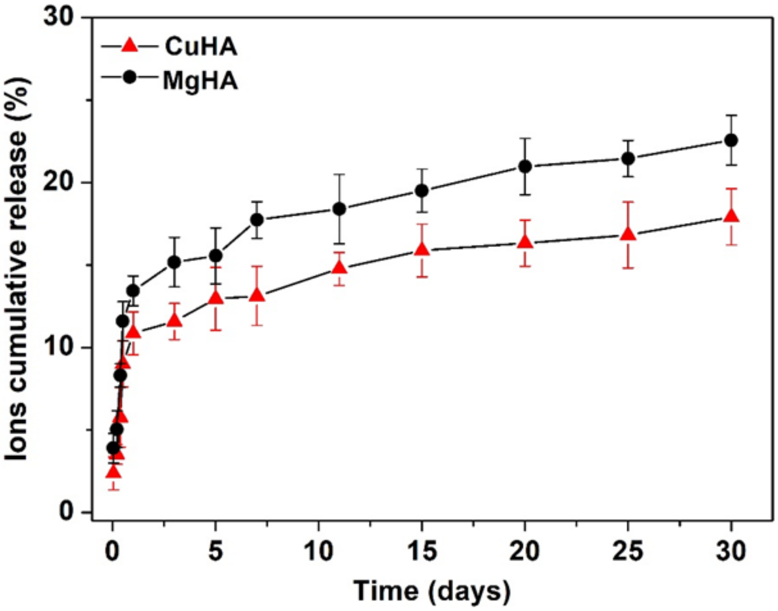

Fig. 3. Release of copper and magnesium ions from HA fiber scaffolds

5. Conclusion

- The release ions assay detects both Cu and Mg ions in the digested PBS over the period, which were degraded from the CuHA and MgHA fiber scaffolds, respectively.
- The copper and magnesium ions released from fiber scaffolds could affect angiogenesis capacity and hasten bone healing and regeneration.

Acknowledgment

This work was supported financially by the National Natural Science Foundation of China (Grant No. 51572228) and Karary University, Khartoum, Sudan.

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