

Resistance of DLP-Fabricated Hydroxyapatite Scaffolds to Ultrashort Electron- Beam and X-ray Irradiation: Structural Stability and Biological Response

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Resistance of DLP-Fabricated Hydroxyapatite Scaffolds to Ultrashort Electron-Beam and X-ray Irradiation: Structural Stability and Biological Response

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Abstract

The effect of ultrashort 3.5 MeV electron-beam (e-beam) and X-ray irradiation on additively manufactured hydroxyapatite (HA) scaffolds was systematically investigated over a low dose range (20–150 Gy). The scaffolds were fabricated using digital light processing (DLP) and subsequently exposed to irradiation conditions relevant to both clinical and processing environments.

The structural and microstructural stability of the samples was examined using X-ray diffraction (XRD) and scanning electron microscopy (SEM). Based on the obtained results, all irradiated samples retained the characteristic HA phase. No noticeable phase transformation, peak shift, or changes in the microstructure were observed within the studied dose range.

The biological behavior of the scaffolds was evaluated in vitro by immersion in simulated body fluid (SBF) and Tris buffer solution. In all cases, similar apatite formation was observed after SBF treatment, and the degradation profiles in Tris were also comparable. Overall, these results suggest that the applied irradiation dosages did not lead to any significant changes in either the bioactivity or the dissolution behavior of the scaffolds.

Overall, no significant differences were observed between non-irradiated and irradiated samples, including both ultrashort e-beam and X-ray treated specimens. These findings demonstrate that DLP-fabricated hydroxyapatite scaffolds exhibit high resistance to ionizing radiation across both clinically relevant and elevated dose ranges, maintaining their structural integrity and biological functionality.

Highlights

- Ultrashort e-beam and X-ray exposure did not alter HA phase or microstructure
- Irradiated scaffolds showed comparable apatite formation in SBF
- Similar Ca and P ion release profiles were observed after irradiation

Key words: Hydroxyapatite; Ultrashort Electron-beam irradiation; X-ray irradiation; Bioceramics; 3D printing; Bioscaffolds; Bioactivity; Biodegradation.

1. Introduction

Bone defects caused by trauma, tumor resection, congenital conditions, or degenerative diseases remain a major clinical challenge and often require biomaterials that can support osteoconduction and provide long-term stability [1]. Hydroxyapatite (HA), is widely used in biomedical applications because of its chemical similarity to the mineral phase of bone and its excellent biocompatibility and bioactivity [2,3]. However, the performance of HA-based implants is strongly influenced by their microstructure, crystallinity, surface morphology, and processing route, which directly affect both degradation behavior and biological response [4].

Additive manufacturing (AM) technologies have significantly expanded the design possibilities for bone scaffolds by enabling the fabrication of patient-specific implants with controlled porosity and complex architectures. Among AM techniques, vat photopolymerization processes such as digital light processing (DLP) provide high resolution and excellent dimensional accuracy. Recent studies have demonstrated that DLP-fabricated hydroxyapatite scaffolds can achieve well-defined porous structures with promising mechanical performance and biological functionality, making them attractive candidates for bone tissue engineering applications [5].

E-beam irradiation is widely used for the sterilization of medical devices due to its rapid processing, precise dose control, and absence of chemical residues. Radiation

sterilization is typically performed at doses in the range of 25–50 kGy, with 25 kGy commonly accepted as the standard dose to ensure sterility of healthcare products [6–8]. From a clinical perspective, e-beams in the MeV energy range are applied in radiotherapy, including techniques such as intraoperative electron radiation therapy (IOERT), where localized doses are delivered to superficial tissues. In conventional radiotherapy, total doses for solid tumors generally fall within the range of 2–80 Gy, delivered in fractionated schedules, while treatments involving superficial malignancies often employ electron beams due to their limited penetration depth. As a result, implanted biomaterials may be exposed to cumulative radiation doses during therapy [9,10].

In the case of HA, previous studies have reported that exposure to an ionizing beam can generate defect structures, such as oxygen vacancies, and induce subtle lattice modifications without necessarily causing macroscopic phase transformations [11]. For instance, Raj et al. [12] demonstrated that continuous MeV e-beam irradiation leads to defect formation within the HA crystal structure while preserving overall phase stability. Notably, ionizing e-beam radiation in this energy range can induce both irradiation-driven (non-thermal) effects—such as point defect generation and electronic excitations—and thermal effects associated with beam energy deposition and localized heating, which may influence surface reactivity, dissolution behavior, and biological interactions. However, the significant thermal load often associated with continuous beams complicates the interpretation of these changes, as it becomes difficult to decouple ionization-induced effects from thermally driven phenomena. To address this, the present study utilizes the AREAL linear accelerator to deliver ultrashort picosecond electron bunches (3.6 MeV), where energy deposition occurs on a timescale significantly faster than the material's thermal relaxation. This allows for the investigation of intrinsic radiation-driven effects within a strictly athermal environment, ensuring that thermal contributions remain negligible even at high cumulative doses [12].

X-ray irradiation is also widely used in clinical and biomedical contexts, particularly in diagnostic imaging and radiotherapy. Unlike electron beams, X-rays interact with materials mainly through indirect ionization processes, including photoelectric absorption and Compton scattering. In calcium phosphate-based materials, these interactions may generate local defects, alter surface chemistry, or induce subtle changes in lattice strain; however, major phase transformations are generally not expected at low clinical doses. Studies on irradiated bone and dentin suggest that X-ray exposure can affect the mineral–

organic interface and mechanical behavior, while the hydroxyapatite mineral phase often remains comparatively stable.

Despite the increasing use of additively manufactured HA scaffolds and the known sensitivity of calcium phosphate materials to irradiation conditions, the response of DLP-fabricated HA scaffolds to low-dose ionizing radiation remains insufficiently understood. Most previous studies have focused either on high-dose sterilization conditions or on biological hard tissues, where the presence of collagen complicates the interpretation of irradiation-induced changes. In contrast, limited information is available on how pure ceramic HA scaffolds produced by DLP respond to clinically relevant doses of ultrashort e-beam and X-ray irradiation in terms of phase stability, microstructure, bioactivity, and dissolution behavior.

In the present study, 3D-printed hydroxyapatite scaffolds were irradiated using a 3.5 MeV ultrashort ($\sim ps$) e-beam generated by the AREAL linear accelerator operated at the CANDLE SRI (Yerevan, Armenia), with typical parameters including picosecond-scale bunch duration, a bunch charge of approximately 200 pC, and a repetition rate of 20 Hz [13–15]. In this regime, due to the extremely short pulse duration and low average power deposition, the irradiation conditions predominantly induce radiation-driven effects, while thermal contributions remain negligible; therefore, the observed modifications can be attributed primarily to irradiation effects without significant thermal influence. For comparison, X-ray radiation was also employed as a reference at equivalent dosage levels. The effect of irradiation over a wide dose range (20–150 Gy) was investigated through surface and structural characterization using scanning electron microscopy (SEM) and X-ray diffraction (XRD), together with biological evaluation including biodegradability and biocompatibility assessments.

The aim of this work is to determine whether low-dose ultrashort e-beam and X-ray irradiation induce measurable structural, microstructural, or biological changes in DLP-fabricated hydroxyapatite scaffolds. Establishing this behavior is important for evaluating the reliability of HA-based implants in radiation-exposed clinical environments and for defining safe irradiation conditions for biomedical applications.

2. Materials and methods

2.1. Additive manufacturing

Three-dimensional hydroxyapatite ceramic specimens with a cylindrical geometry (diameter 8.3 mm, height 0.7 mm) were fabricated using a DLP-based system (CeraFab Lab L30, Lithoz). Printing was performed using a commercial slurry (LithaBone HA 483, Lithoz, Austria), specifically developed for vat photopolymerization.

The feedstock consists of homogeneously dispersed hydroxyapatite particles within a photocurable organic resin based on acrylate/methacrylate monomers, combined with a photoinitiator and suitable additives to ensure stable dispersion and appropriate rheological behavior.

The printing process was carried out using a blue LED light source (450–460 nm). A layer thickness of 25 μm was applied, with an exposure energy of 300 mJ/cm^2 and corresponding light intensity of 58.4 mW/cm^2 . To improve dimensional accuracy, a contour correction of $-50\text{ }\mu\text{m}$ was implemented. Additionally, scaling factors of 1.34 (x, y) and 1.31 (z) were applied to compensate for shrinkage during thermal processing. The complete set of printing parameters is summarized in Table 1.

Table 1. Printing parameters used for DLP fabrication.

Parameter	Value
Light source	Blue LED
Wavelength	450–460 nm
Light intensity	58.4 mW/cm^2
Exposure energy	300 mJ/cm^2
Layer thickness	25 μm
Contour correction	$-50\text{ }\mu\text{m}$
Scaling factor (X, Y)	1.34

After fabrication, the printed green bodies were carefully removed from the build platform to avoid structural damage. The samples were cleaned using LithoSol 80 solvent, followed by ultrasonic treatment to ensure complete removal of residual slurry.

The cleaned and dried specimens were then subjected to a combined debinding and sintering process (Fig. 1) in an electric furnace (MLR 18-12L, Termolab, Portugal), following the manufacturer's recommended thermal profile. The total duration of the heat treatment was approximately 122 hours.

In vitro bioactivity was evaluated by immersion of the samples in simulated body fluid (SBF) [6] at 37 °C for periods of 1, 2, and 4 weeks. The samples had an average mass of 0.11 g and an average surface area of 126.46 mm². Each specimen was immersed in 13 mL of SBF solution. The solution was renewed every 48 h to maintain ionic stability. After each immersion period, the samples were removed, rinsed with deionized water, and dried prior to characterization.

Biodegradability was assessed by immersion in Tris–HCl buffer solution (pH 7.4) for periods of 1 day, 1 week, 1 month, and 3 months. Each sample was immersed in 13 mL of solution.

All experiments were conducted at 37 °C under continuous agitation (110 rpm) using a laboratory shaker (Rotabit, J.P. Selecta S.A., Spain).

2.4. Characterization

Phase characterization was accomplished by X-ray diffraction (Rigaku MiniFlex 600 X-ray diffractometer, 40 mA, 40 kV, CuK α radiation, λ = 0.1542nm, step size of 0.02°). Microstructural characterization was performed by scanning electron microscopy (SEM, Prisma E, Thermo Fisher Scientific, and Hillsboro, OR, USA). Samples were coated with a 20nm layer of Au to provide sufficient conductivity. The concentrations of calcium (Ca) and phosphorus (P) ions released into Tris-HCl solutions were measured using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) IRIS ADVANTAGE from Termo Jarrel Ash (USA).

3. Results

3.1. Phase composition and microstructure

The phase composition of the non-irradiated and irradiated hydroxyapatite samples was examined by XRD, and the corresponding patterns are presented in Fig. 2.

All samples, including non-irradiated, ultrashort e-beam and X-ray irradiated specimens (20, 50, 70, 100, 150 Gy, as well as 1 and 2 kGy), exhibited diffraction peaks characteristic of hydroxyapatite, in good agreement with the reference pattern (JCPDS No. 9-432). No additional phases, such as tricalcium phosphate (TCP), were detected after irradiation. Furthermore, no measurable peak shifts, peak broadening, or changes in relative intensities were observed.

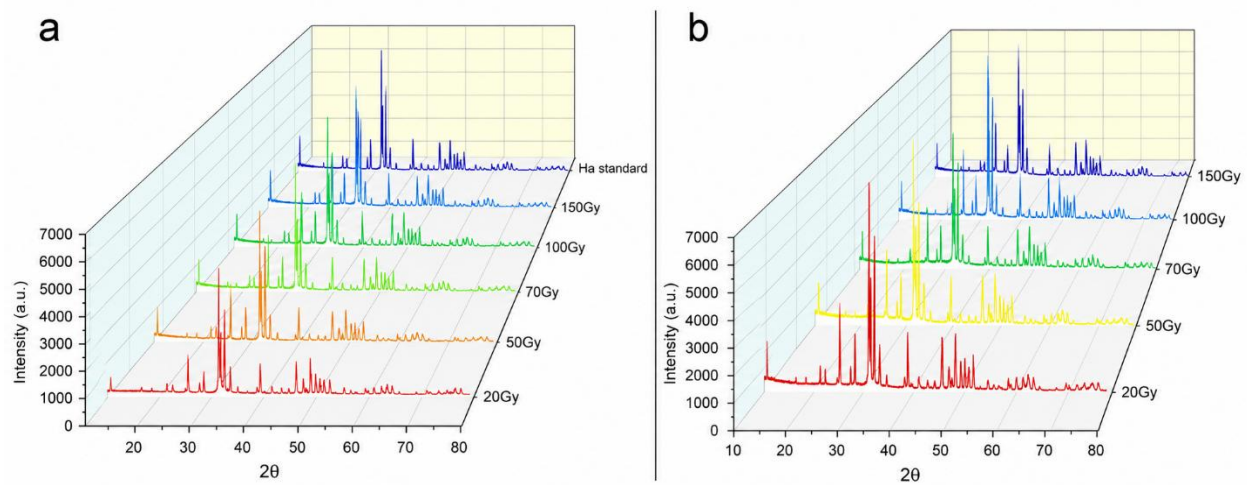


Fig. 2. XRD patterns of the samples before and after ultrashort e-beam irradiation.

The microstructure of the HA scaffolds before and after irradiation was evaluated by SEM, and the corresponding images are shown in Figure 3. The non-irradiated samples exhibited a relatively dense microstructure with well-defined grains and distinct grain boundaries (Fig. 3. (a-c)). Following both ultrashort e-beam (Fig. 3. (d-i)) and X-ray irradiation (Fig. 3. (j-o)), the overall morphology of the samples remained largely unchanged.

For clarity, representative SEM images of the samples irradiated with 20 and 150 Gy are presented in Figure 3. Samples exposed to intermediate irradiation doses of 50, 70, and 100 Gy were also examined and showed microstructures similar to those observed for the presented samples. No noticeable grain degradation, microcrack formation, surface melting, or other irradiation-related defects were observed within the investigated dose range.

Overall, the SEM analysis suggests that both ultrashort e-beam and X-ray irradiation did not produce detectable microstructural changes in the HA scaffolds under the applied experimental conditions. These observations are consistent with the XRD results, which likewise confirmed the preservation of the characteristic HA phase after irradiation.

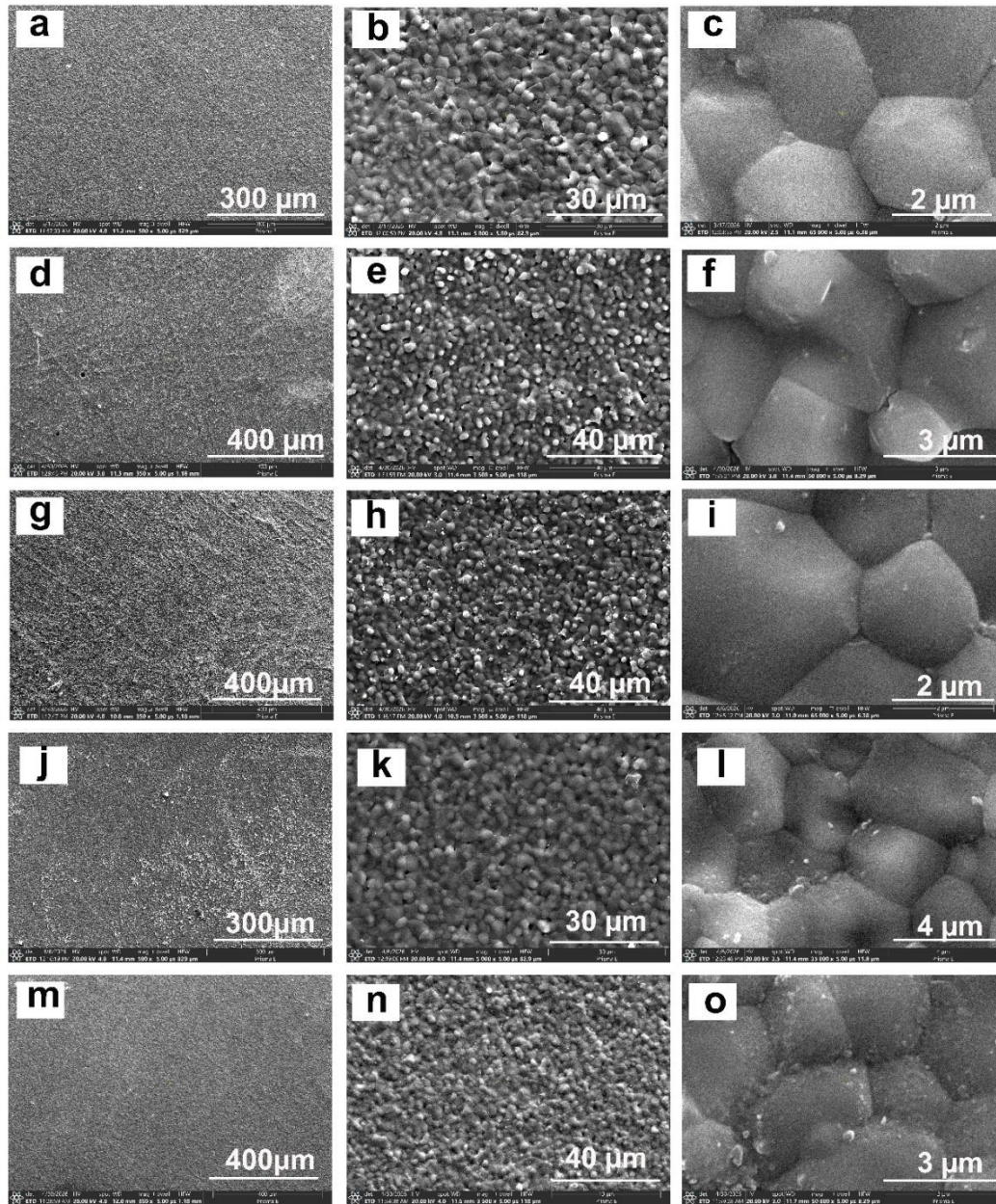


Fig. 3. Microstructure of HA scaffolds before irradiation ((a)–(c)) and after ultrashort e-beam irradiation at doses of 20 Gy ((d)–(f)) and 150 Gy ((g)–(i)), as well as after X-ray irradiation at doses of 20 Gy ((j)–(l)) and 150 Gy ((m)–(o)).

3.2 *in vitro* experiments

The *in vitro* bioactivity of the scaffolds was evaluated before (Fig. 4(a-f)) and after ultrashort e-beam (Fig. 4(d-r)) and X-ray irradiation (Fig. 4a-f) by immersion in SBF for 1, 2, and 4 weeks.

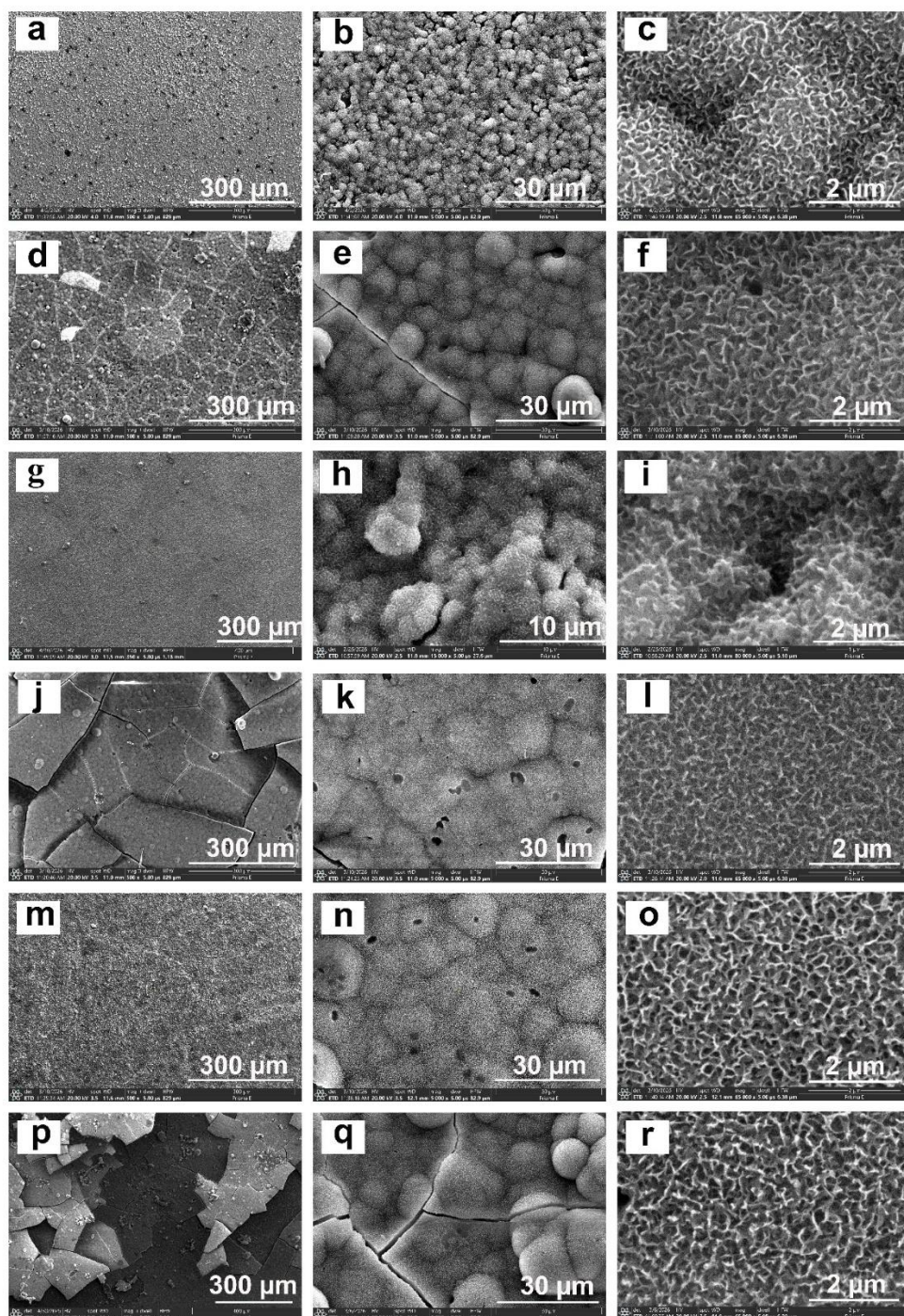


Fig. 4. Microstructure of HA scaffolds after immersion in SBF for 1 week ((a)–(c)) and 4 weeks ((d)–(f)) before irradiation, as well as after ultrashort e-beam irradiation at doses of 20 Gy following 1 week ((g)–(i)) and 4 weeks ((j)–(l)) of immersion, and 150 Gy following 1 week ((m)–(o)) and 4 weeks ((p)–(r)) of immersion in SBF.

After 1 week of immersion (Fig. 4(a-c)), the surface of the non-irradiated samples was partially covered by newly formed apatite deposits. With increasing immersion time, the amount of precipitated layer gradually increased, resulting in a more continuous and dense surface coverage after 4 weeks (Fig. 4(d-f)).

A similar behavior was observed for the irradiated samples. The scaffolds exposed to ultrashort e-beam irradiation at doses of 20 Gy and 150 Gy (Fig. 4(g-r)). Results showed the formation of apatite-like structures already after 1 week of immersion. Further immersion for 2 and 4 weeks led to a progressive increase in the deposited layer. Samples irradiated with intermediate doses exhibited similar surface morphology and apatite formation behavior during SBF immersion.

SEM micrographs of X-ray irradiated samples after one month of SBF immersion at doses of 20 Gy and 150 Gy are presented in Fig. 5. At lower magnification (Fig. 5 (a, d)), both samples exhibited a continuous surface layer with several visible crack-like features distributed across the surface. Similar cracks were observed in both irradiation conditions without a clear dose-dependent trend. At higher magnifications (Fig. 5 (b, c, e, f)), the surfaces showed a characteristic apatite-like morphology composed of interconnected precipitated structures. No substantial differences in surface morphology or apatite coverage were detected between the investigated doses. Since comparable crack features were observed irrespective of irradiation dose and type, they may originate from surface layer formation after immersion.

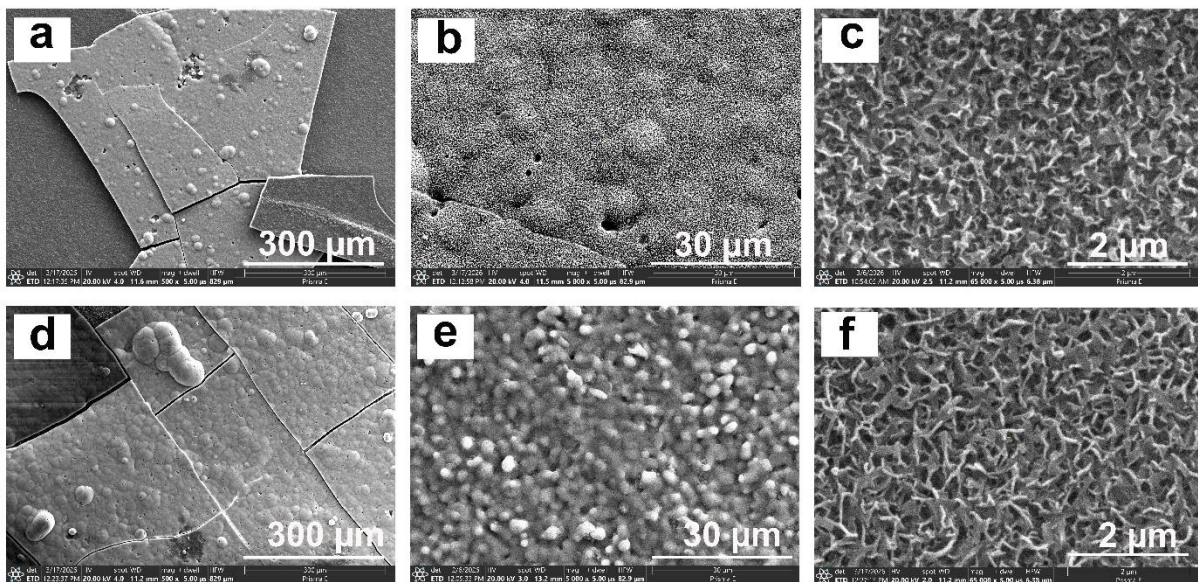


Fig. 5. Microstructure of the HA scaffolds after immersion in SBF for 4 weeks after X-ray irradiation at doses of 20 Gy ((a)–(c)) and 150 Gy ((d)–(f)).

The biodegradation behavior was assessed separately in Tris buffer over the same time intervals (1, 2, and 4 weeks). All samples showed a gradual and comparable degradation trend over time. No significant differences in surface morphology, dissolution behavior, or degradation rate were detected between irradiated and non-irradiated samples Fig. 6. For the ultrashort e-beam irradiated samples, Ca concentrations increased from approximately 1.5 after 1 week to around 6.0–6.9 after 4 weeks. A similar tendency was observed for the X-ray irradiated samples, where Ca release also increased with immersion time and remained close to the values measured for the non-irradiated samples. The P concentrations were much lower than those of Ca, but followed the same general time-dependent trend.

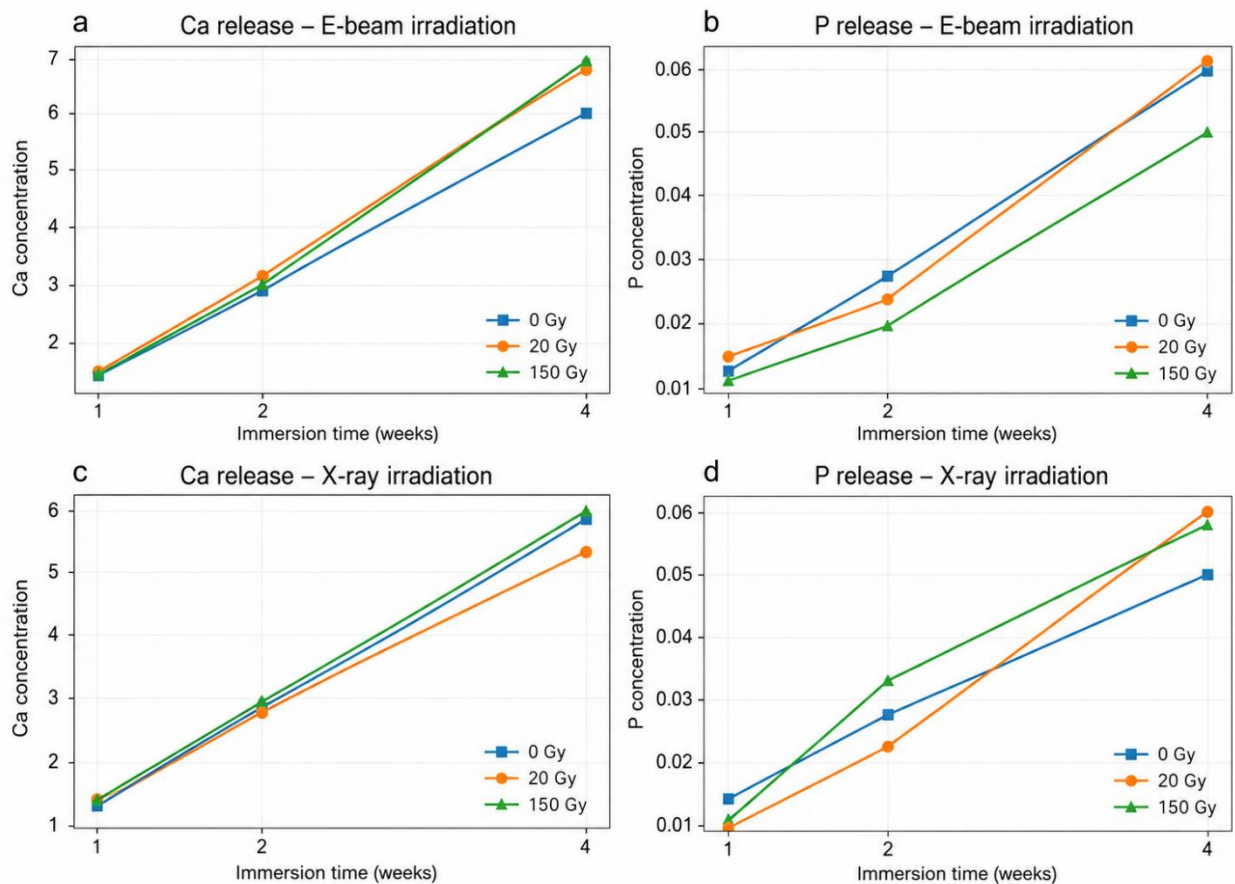


Fig. 6. Calcium and phosphorus ion concentrations released from HA scaffolds after immersion in Tris buffer for 1, 2, and 4 weeks: (a) Ca release after ultrashort e-beam

irradiation, (b) P release after ultrashort e-beam irradiation, (c) Ca release after X-ray irradiation, and (d) P release after X-ray irradiation.

Importantly, no clear dose-dependent difference was observed between the non-irradiated scaffolds and those irradiated at 20 or 150 Gy. The intermediate irradiation doses also showed comparable behavior. These results suggest that, within the investigated dose range, neither ultrashort e-beam nor X-ray irradiation significantly affected the dissolution behavior of the HA scaffolds in Tris buffer. Combined with the SEM observations after SBF immersion, this indicates that the applied irradiation dosages did not negatively influence either the bioactivity or the degradation performance of the scaffolds.

4. Discussion

The results obtained in this study show that HA scaffolds remain structurally and biologically stable after both short pulse e-beam and X-ray irradiation across the investigated dose range (20, 50, 70, 100, 150 Gy).

XRD analysis confirmed that the HA phase is preserved, with no detectable changes in peak position or intensity. This agrees with the generally high radiation resistance of hydroxyapatite, where irradiation effects are often limited to point defects rather than bulk structural changes.

SEM observations also indicate that the scaffold microstructure is not affected by irradiation. No signs of cracking, surface degradation, or changes in pore architecture were detected, even at the highest doses.

It is critical to highlight that the ultrashort e-beam irradiation parameters employed in this work operate within a thermal regime. Due to the ultra-short pulse duration and restricted heat transfer, energy deposition occurs on a timescale significantly faster than the material's thermal relaxation. Consequently, the localized energy density does not reach the activation thresholds required for thermally driven phenomena, such as grain boundary migration (grain growth) or polymorphic phase transformations. The absence of microstructural changes is therefore fundamentally consistent with the decoupled relationship between radiation dose and thermal load inherent to this irradiation method. The bioactivity results further support these observations. All samples showed similar apatite formation in SBF, suggesting that the surface properties responsible for nucleation remain unchanged.

In addition, the degradation behavior measured in Tris buffer was comparable for all samples. Since Tris provides a direct assessment of dissolution without re-precipitation effects, this indicates that irradiation does not influence the intrinsic degradation mechanism of hydroxyapatite.

Overall, the results suggest that any irradiation-induced effects are limited to the atomic scale and do not affect the material

Conclusion

In this study, the effect of short pulse e-beam and X-ray irradiation on DLP-fabricated hydroxyapatite scaffolds was evaluated over a wide dose range (20 Gy to 150 Gy). The results show that irradiation does not induce measurable changes in phase composition or microstructure, as confirmed by XRD and SEM analyses.

In vitro testing further demonstrated that both bioactivity and biodegradation behavior remain unchanged after irradiation. All samples exhibited comparable apatite formation in simulated body fluid and similar dissolution behavior in Tris buffer, indicating that the key functional properties of hydroxyapatite are preserved.

These findings suggest that hydroxyapatite scaffolds possess a high resistance to ionizing radiation, maintaining their structural integrity and biological performance across both clinically relevant and elevated dose levels. This stability is consistent with the non-thermal nature of the applied irradiation dosages, where no significant heat-induced transformations are expected.

Overall, the results support the safe use of hydroxyapatite-based scaffolds in applications where exposure to radiation may occur, such as sterilization or radiotherapy, and define a broad range of irradiation conditions under which their properties remain stable.

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