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Design and Evaluation of Bioactive Glass/Polymer Hybrid Scaffolds Loaded with Methotrexate for bone tissue engineering

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This study reports the fabrication and evaluation of a novel hybrid scaffold developed as a potential platform for localized and sustained drug delivery, to be confirmed in future release studies. The scaffold integrates bioactive glass with a polymeric network composed of polyvinyl alcohol (PVA), cellulose nanocrystals (CNC), and sodium alginate (SA). Methotrexate (MTX) was incorporated into the PVA–CNC–SA matrix reinforced with a fixed amount (0.4 g) of bioactive glass (65% SiO_2 –30% CaO–5% P_2 O_5) using the freeze-drying technique, resulting in a single optimized formulation with a highly porous and bioactive architecture suitable for bone tissue engineering. The developed scaffold was comprehensively characterized using FTIR, XRD, SEM, and EDXA analyses. Its bioactivity was confirmed through immersion in simulated body fluid (SBF), where the formation of a hydroxyapatite layer indicated strong biological reactivity. Although quantitative drug-release experiments were not performed at this stage, the system was conceptually designed to achieve controlled methotrexate delivery. Overall, the findings demonstrate the potential of the optimized freeze-dried hybrid scaffold as a multifunctional platform capable of supporting bone regeneration while enabling localized chemotherapy applications.

Keywords: Drug carrier, PVA, CNC, Bioactivity, Bioglass, Scaffold.

Introduction

Advances in biomaterials have opened new opportunities for tackling the persistent challenges of cancer therapy. Among these, multifunctional scaffolds that integrate structural support with localized drug delivery are gaining attention for their potential to regenerate tissue while simultaneously inhibiting tumor growth. Such systems provide controlled and sustained release of chemotherapeutic agents, thereby improving therapeutic efficiency and minimizing systemic toxicity [1], [2] .

Bioactive glass (BG) has been widely recognized as a key component in this approach due to its strong bioactivity, direct bonding ability with bone tissue, and modifiable surface chemistry that allows drug loading and functionalization [3], [4]. When combined with polymeric matrices, BG not only reinforces mechanical strength but also enhances osteoconductivity and mineralization.

Polymeric carriers offer versatility in engineering drug delivery systems. Polyvinyl alcohol (PVA) is widely employed because of its mechanical robustness, hydrophilicity, and biocompatibility [5]. However, its relatively low water resistance limits performance, which has encouraged the development of hybrid systems. Incorporating sodium alginate (SA) improves structural stability [6], while cellulose nanocrystals (CNCs) contribute mechanical reinforcement and increase reactive surface area for functionalization [7], [8]. Together, these polymers provide a synergistic platform for bone tissue engineering and targeted chemotherapy.

One of the key clinical drivers for such innovations is osteosarcoma, the most prevalent malignant primary bone tumor. Despite surgical advancements and chemotherapeutic regimens, overall survival remains below 60%, and treatment is frequently accompanied by drug resistance and systemic side effects [9], [10]. Methotrexate (MTX) continues to play a central role, but its poor aqueous solubility, rapid clearance, and dose-limiting toxicities highlight the urgent need for improved delivery platforms [11], [12].

Despite extensive studies on polymeric and nanoparticulate MTX carriers, most existing systems face challenges in achieving localized, sustained delivery at bone defect sites. In contrast, bioactive glass/polymer hybrid scaffolds combine osteoconductivity with drug-carrying capability, enabling dual functionality—bone regeneration and localized chemotherapy—which represents the core innovation of this study.

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In the present study, a single optimized hybrid scaffold formulation composed of PVA, CNC, SA, and bioactive glass (65% SiO_2–30% CaO–5% P_2O_5) was fabricated using the freeze-drying technique. The developed scaffold was evaluated for its physicochemical characteristics, structural stability, and in vitro bioactivity through immersion in simulated body fluid (SBF). This work aims to demonstrate the potential of the optimized freeze-dried hybrid scaffold as a multifunctional platform that supports bone regeneration and offers a promising foundation for localized methotrexate delivery in osteosarcoma therapy.

Material and methods

Materials:

Tetraethyl orthosilicate (TEOS, $C_8 H_{20} O_4 Si$, Mw 208.33 g/mol), calcium nitrate tetrahydrate ($Ca(NO_3)_2 \cdot 4H_2O$, Mw 236.15 g/mol), triethyl phosphate (TEP, $C_6 H_{15} O_4 P$, Mw 182.15 g/mol), and 2 M nitric acid (HNO_3) were obtained from Merck Inc. (Darmstadt, Germany). Acetic acid (96%), sodium alginate (SA, NaAlg), polyvinyl alcohol (PVA, $(C_2 H_4 O)$□, and Cellulose nanocrystals (CNC) were purchased from Acros Organics Ltd. (New Jersey, USA). Methotrexate (MTX, $C_{20} H_{22} N_8 O_5$, Mw 454.45 g/mol) was sourced from SPH Sine Pharmaceutical Laboratories Co. Ltd. (H3102067804, Shanghai, China). All other reagents needed for preparing simulated body fluid (SBF) and phosphate-buffered saline (PBS) were acquired from Sigma-Aldrich (St. Louis, MO, USA).

Synthesis of bioactive glass (65S-BG)

Bioactive glass with a molar composition of 65% SiO_2, 30% CaO, and 5% P_2O_5 (65S-BG) was synthesized using the sol–gel method as previously described [13], [14], [15]. Briefly, tetraethyl orthosilicate (TEOS) was hydrolyzed in 2 M nitric acid under stirring for 30 min. Triethyl phosphate (TEP) and calcium nitrate tetrahydrate ($Ca(NO_3)_2 \cdot 4H_2O$) were then added sequentially, each with an additional 45 min of stirring. The resulting sol was continuously stirred to complete hydrolysis, dried at 120 °C for 3 days, and subsequently calcined at 600 °C for 3 h to remove nitrate residues and incorporate calcium into the silicate network [16]. The obtained powder was ground and sieved to <90 µm for further use.

Synthesis of MTX/BG loaded PVA-CNC-SA biocomposite scaffold

MTX/BG-loaded PVA-CNC-SA biocomposite scaffold was prepared using a modified sol–gel and via the freeze-drying technique [17], [18] . Briefly, sodium alginate (SA, 1 g) and polyvinyl alcohol (PVA, 0.6 g) were dissolved in 35 mL of deionized water under stirring (Solution A). Methotrexate (MTX, 0.6 g) and bioactive glass (BG, 0.4 g) were dispersed in deionized water and stirred for 1 h (Solution B). Cellulose nanocrystals (CNC, 1 g) were separately dispersed for 30 min (Solution C).

The three solutions were then combined and homogenized at 969 rpm using a high-torque digital overhead stirrer (HT-50DX, Germany) for 2 h to obtain a uniform mixture. In this work, the MTX/BG-loaded PVA-CNC-SA scaffolds were frozen at –80 °C or by immersion in liquid nitrogen prior to lyophilization, resulting in structures with well-developed porosity and interconnected cavities.

Table 1. Chemical composition of MTX/BG loaded PVA-CNC-SA Scaffold in (wt %).

Sample Code	PVA (g)	CNC (g)	SA (g)	BG (g)	MTX (g)
D2	0.6	1	1	0.4	0.6

In vitro bioactivity study

The bioactivity of MTX/BG-loaded PVA-CNC-SA scaffolds was evaluated by immersion in simulated body fluid (SBF), following the protocol described by [19]. Briefly, 1 g of the scaffold was placed in a sealed container with 40 mL of SBF and incubated at 37 °C for 30 days. This solid-to-liquid ratio (1:40 g/mL) corresponds to the standard value of 0.025 g/mL recommended by Kokubo & Takadama to ensure comparable ion exchange conditions.

Characterization

The MTX/BG-loaded PVA-CNC-SA scaffolds, both before and after immersion in SBF, were characterized using multiple analytical techniques. X-ray diffraction (XRD) patterns were recorded on a Bruker D8 ADVANCE diffractometer (Germany) equipped with a Cu Kα radiation source (λ = 1.54060 Å) and a nickel filter, over a 2θ range of 0–60°, with a step size of 0.014° and a counting time of 1 s per step. Fourier-transform infrared (FTIR) spectra were obtained using a Nicolet 6700 spectrometer (Thermo Scientific, USA). Surface morphology was examined by field emission scanning electron microscopy (FEG-SEM; Philips XL30), while elemental composition was determined using energy-dispersive X-ray spectroscopy (EDX; 30 mm² Si(Li) R-RSUTW detector) operated at an accelerating voltage of 15 Kv.

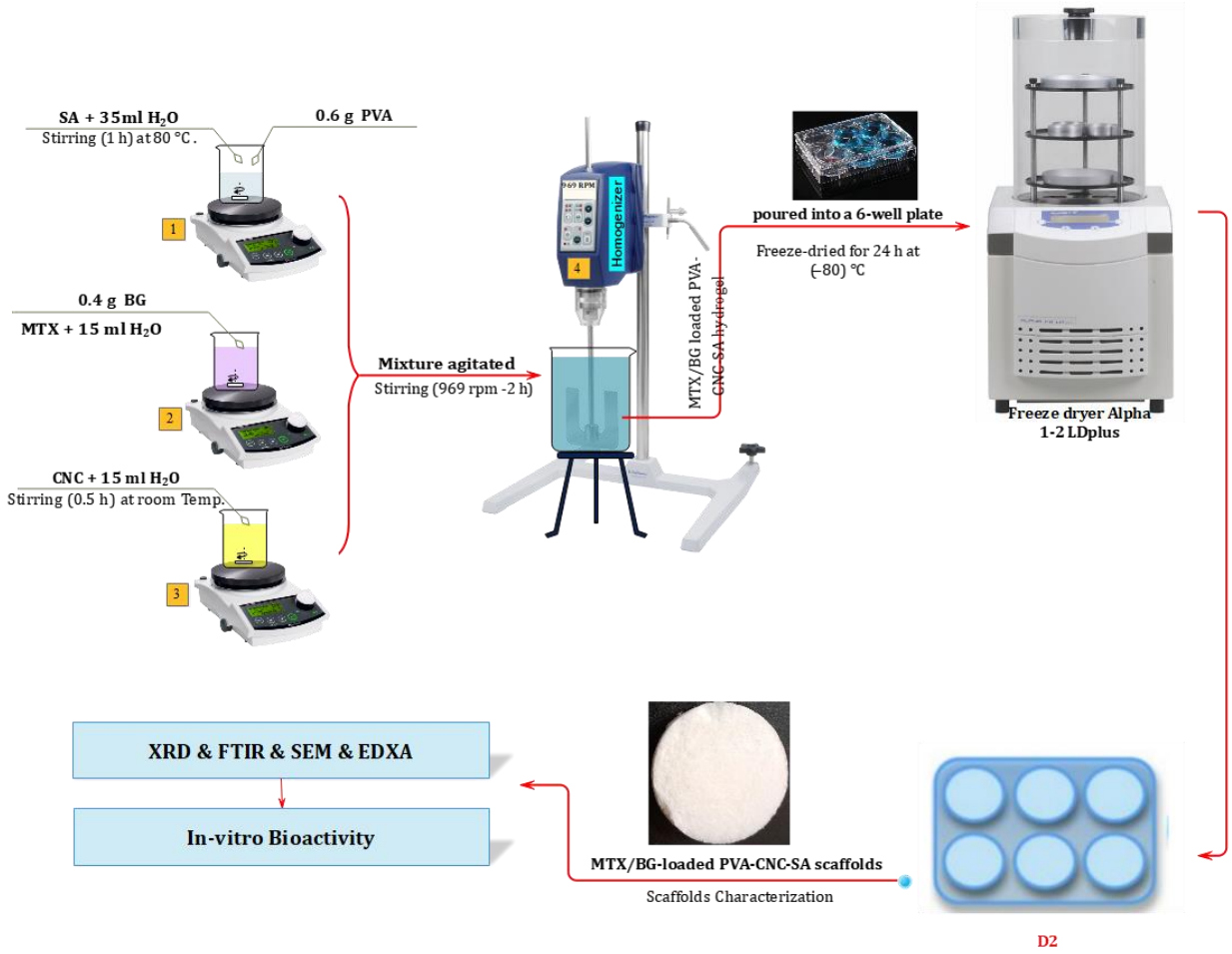

Fig. 1. Flow chart of the synthesis of MTX/BG loaded PVA-CNC-SA composites scaffold.

Results and discussion:

XRD of the biocomposite Scaffold Before and After SBF

Figure 2 presents the XRD patterns of MTX/BG-loaded PVA-CNC-SA scaffolds before and after immersion in SBF for 30 days. The broad diffraction halo at ~22.5° confirmed the amorphous nature of the scaffold, consistent with the presence of bioactive glass within the polymeric matrix. This amorphous phase is advantageous for bioactivity, as it facilitates the rapid exchange of ions (Ca^{2+} , Si^{4+} , and PO_4^{3-}) between the scaffold and the surrounding medium, accelerating hydroxyapatite nucleation on the surface.

Thus, the structural disorder of the glassy phase contributes directly to the enhanced biological reactivity observed in SBF.

Following immersion in SBF, three characteristic peaks emerged at approximately $2\theta \approx 32°$, which can be attributed to the formation of hydroxyapatite (HA), as referenced in the JCPDS card No. 09-0432 [20]. These findings demonstrate the bioactivity of the scaffolds through apatite layer formation.

FTIR of the biocomposite Scaffold

Figure 3 shows the FTIR spectra of the MTX/BG/PVA/CNC/SA biocomposite scaffold before and after immersion in simulated body fluid (SBF) for 30 days. A broad band at ~3419 cm^{-1} is attributed to the stretching vibrations of –OH and –NH groups, while the band at 2923 cm^{-1} corresponds to the C–H stretching of aliphatic groups in the polymeric components. Characteristic bands at ~1620 and ~1442 cm^{-1} are assigned to amide I and amide II vibrations, respectively, which are related to methotrexate and the biopolymer matrix. The strong peak at ~1070–1085 cm^{-1} represents Si–O–Si asymmetric stretching of the bioactive glass, whereas the bands at 600–560 cm^{-1} correspond to P–O vibrations of phosphate groups.

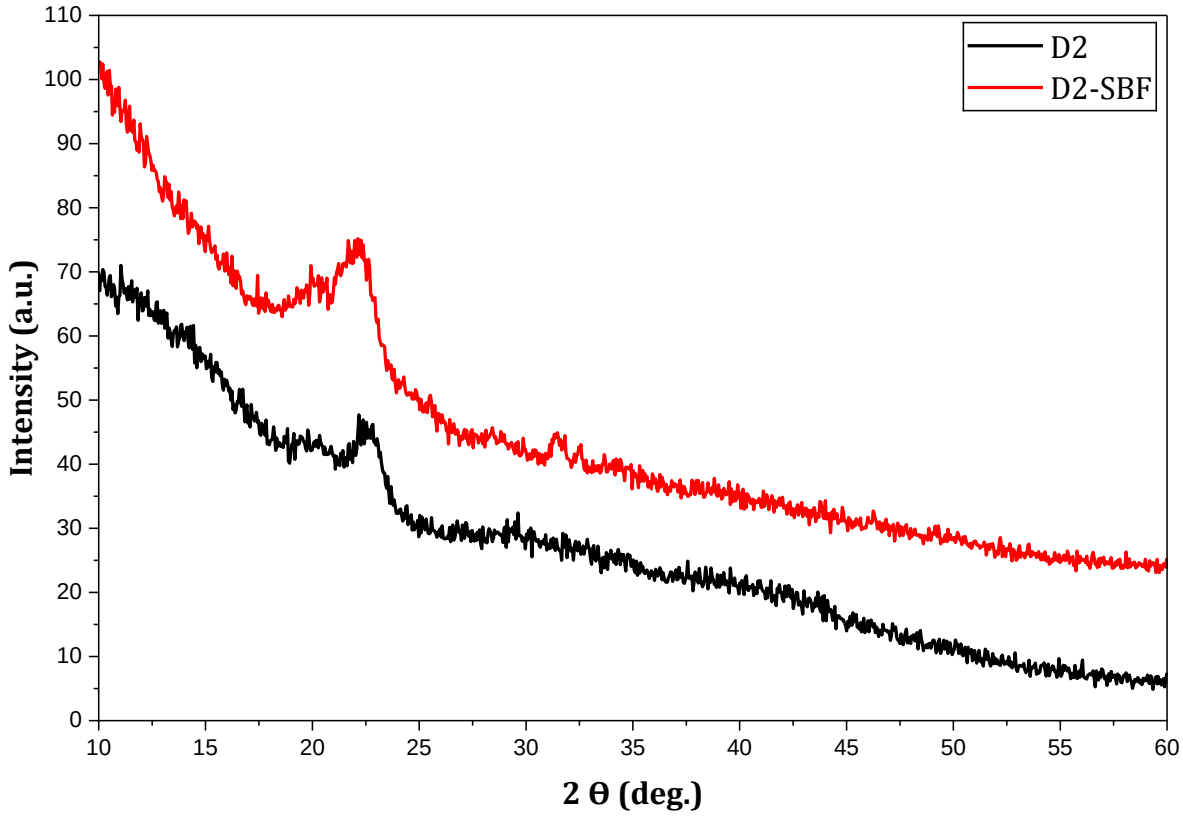

Fig. 2. (XRD) patterns of MTX, BG, PVA, CNC, and SA biocomposite Scaffold before and After immersion in SBF for 30 days.

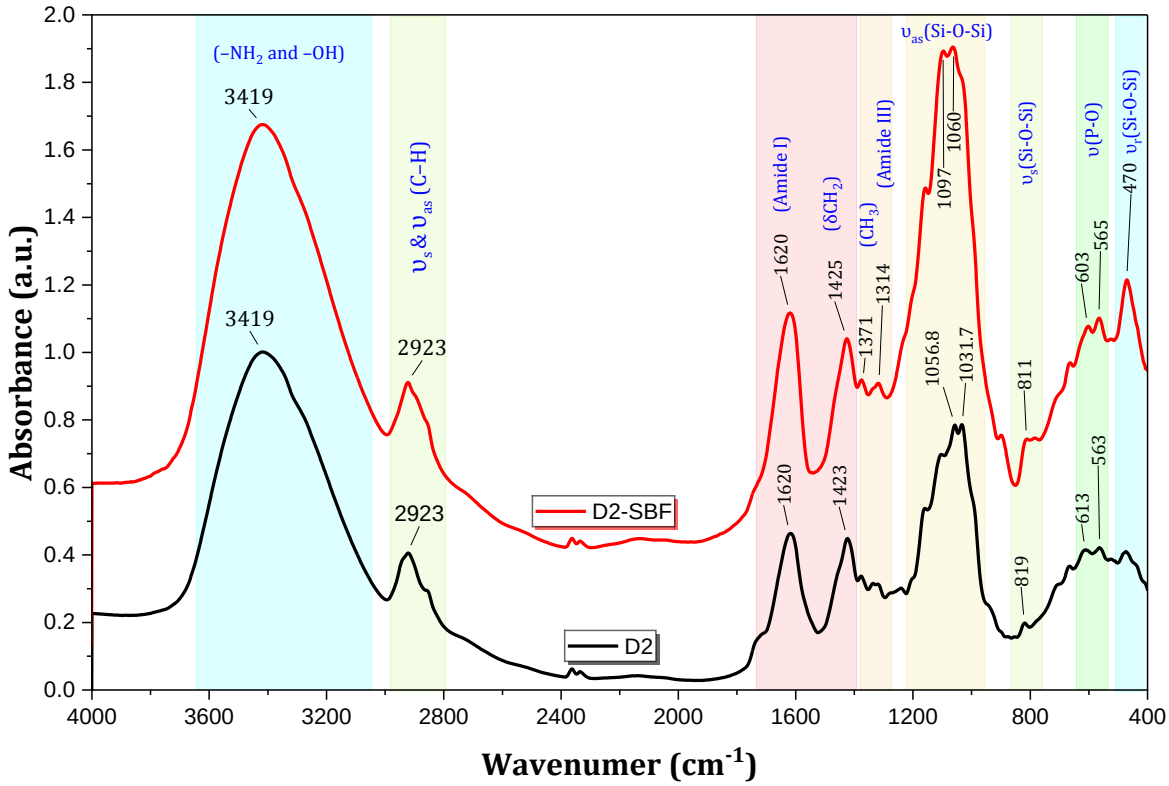

Fig. 3. (FTIR) patterns of MTX, BG, PVA, CNC, and SA biocomposite Scaffold before and After immersion in SBF for 30 days.

The marked increase in phosphate band intensity (at ~566 and ~603 cm^{-1}) after SBF immersion confirms the deposition of phosphate-containing mineral phases, primarily hydroxyapatite. The interaction between silanol (Si–OH) groups from the glass phase and Ca^{2+} ions from SBF promotes apatite nucleation. This chemical bonding illustrates how the scaffold composition directly supports mineralization processes and enhances bioactivity.

SEM & EDXA

Figure 4. High-resolution **SEM** micrographs of the optimized PVA–CNC–SA/BG scaffold before (a) and after (b) immersion in SBF for 30 days. The images clearly show the porous structure and apatite deposition (scale bars: 300 & 40 μm).

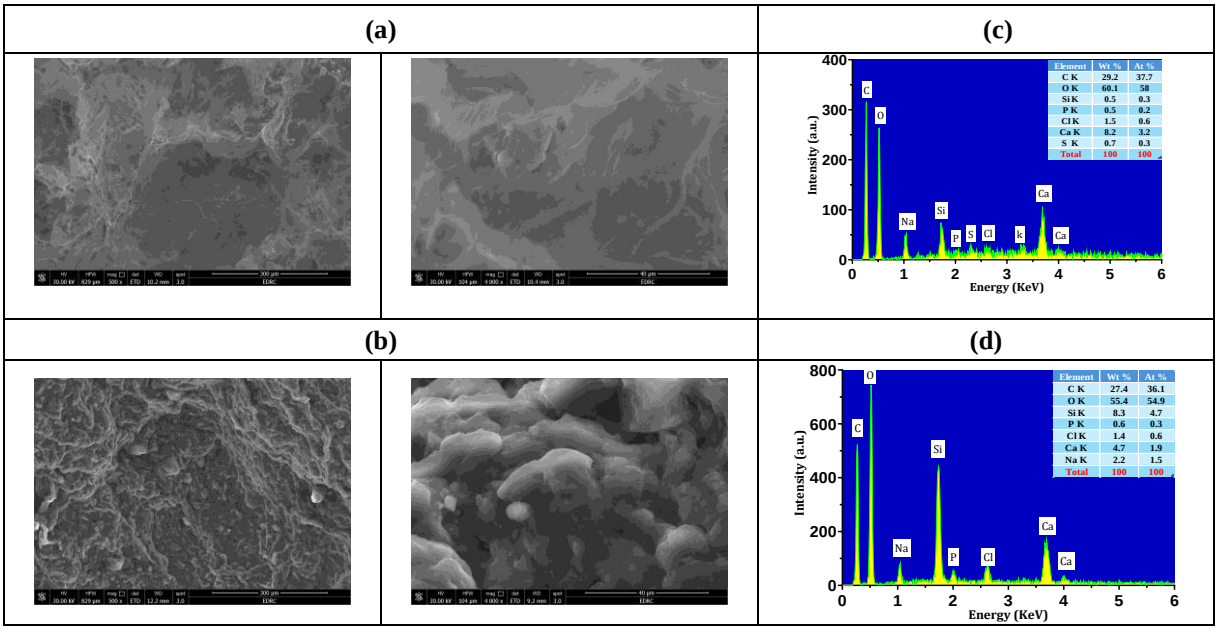

Fig. 4. Scanning Electron Microscopy (SEM) images of, (a) D1 Before immersion in SBF, (b) D1 After immersion in SBF for 30 days, and EDXA profiles of (c) D1 Before immersion in SBF and (d) D1 After immersion in SBF for 30 days.

The surface morphology evolved from a smooth to a rough and granular texture *after SBF immersion* (Figure 4b), consistent with apatite layer formation. The observed globular particles represent early nucleation sites that coalesce into a continuous mineral layer, providing evidence of strong scaffold–medium interaction. Such surface mineralization is essential for establishing direct bonding with bone tissue and validating the scaffold's bioactive behavior. The **EDXA** profile before immersion (Figure 4c) mainly showed signals corresponding to Si, C, and O, originating from the bioactive glass and polymeric components. In contrast, after immersion (Figure 4d), distinct peaks of Ca and P appeared, and the Ca/P atomic ratio approached that of hydroxyapatite, confirming the nucleation and growth of a calcium phosphate layer on the scaffold surface. These morphological and compositional changes provide strong evidence of the bioactivity of the scaffold and its ability to induce apatite formation in a physiological-like environment

Limitations and Future Work

Although this study successfully demonstrated the fabrication and physicochemical characterization of the MTX/BG-loaded PVA–CNC–SA hybrid scaffold, certain limitations should be acknowledged.

First, in vitro cytocompatibility and quantitative drug-release assessments were not performed at this stage. These experiments are crucial to verify the therapeutic efficacy and controlled-release performance of the scaffold.

Second, mechanical testing such as compressive strength and elastic modulus measurements was not carried out, which would provide further insight into the scaffold's mechanical integrity and suitability for load-bearing bone applications.

In future work, biological assays involving osteoblast and osteosarcoma cell lines, together with mechanical evaluations, will be conducted to establish a more comprehensive understanding of the scaffold's biomedical functionality and performance. Additionally, comparative studies employing alternative polymeric matrices such

as chitosan or gelatin will be explored to evaluate the influence of polymer composition on scaffold properties and bioactivity

Conclusions

In this work, an MTX/BG-loaded PVA–CNC–SA hybrid scaffold was successfully fabricated via the freeze-drying technique and comprehensively characterized using FTIR, XRD, SEM, and EDXA analyses. The incorporation of bioactive glass significantly enhanced the scaffold's bioactivity, as evidenced by the formation of hydroxyapatite confirmed through both structural and morphological analyses. Upon immersion in simulated body fluid (SBF), the scaffold exhibited clear morphological and compositional changes including apatite layer deposition and Ca/P ratios consistent with hydroxyapatite validating its capacity to stimulate biomineralization.

While quantitative drug-release measurements were not performed, the composite design indicates potential for localized and sustained methotrexate delivery, to be confirmed in future studies. These integrated features highlight the promising dual functionality of the developed scaffold in promoting bone regeneration while providing controlled chemotherapeutic release.

Overall, the results demonstrate that the optimized freeze-dried PVA–CNC–SA scaffold incorporating bioactive glass and methotrexate represents a promising multifunctional system for future applications in bone tissue engineering and regenerative medicine.

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Ethical of approval: This study follows the ethics guidelines .

References

[1] J. Chen, X. Chen, Z. Yang, X. Tan, J. Wang, and Y. Chen, "Preparation and characterization of folic acid functionalized bioactive glass for targeted delivery and sustained release of methotrexate," *J Biomed Mater Res A*, vol. **107**, no. 2, pp. 319–329, Feb. (2019).

[2] R. Borges et al., "Bioactive Glasses as Carriers of Cancer-Targeted Drugs: Challenges and Opportunities in Bone Cancer Treatment," *Materials*, vol. **15**, no. 24, Dec. (2022).

[3] A. Lukowiak, J. Lao, J. Lacroix, and J. Marie Nedelec, "Bioactive glass nanoparticles obtained through sol–gel chemistry," *Chemical Communications*, vol. **49**, no. 59, pp. 6620–6622, Jun. (2013).

[4] M. Diani, V. Grasso, and G. Altomare, "Methotrexate: practical use in dermatology.," *G Ital Dermatol Venereol*, vol. **151**, no. 5, pp. 535–543, Jan. 2016, Accessed: Jun. 14, (2024).

[5] S. Das and U. Subuddhi, "Controlled delivery of ibuprofen from poly(vinyl alcohol)−poly(ethylene glycol) interpenetrating polymeric network hydrogels," *J Pharm Anal*, vol. **9**, no. 2, pp. 108–116, Apr. (2019).

[6] H. Anwar, M. Ahmad, M. U. Minhas, and S. Rehmani, "Alginate-polyvinyl alcohol based interpenetrating polymer network for prolonged drug therapy, Optimization and in-vitro characterization," *Carbohydr Polym*, vol. **166**, pp. 183–194, Jun. (2017).

[7] R. J. Moon, A. Martini, J. Nairn, J. Simonsen, and J. Youngblood, "Cellulose nanomaterials review: structure, properties and nanocomposites," *Chem Soc Rev*, vol. **40**, no. 7, pp. 3941–3994, Jun. (2011).

[8] H. N. Xu, Y. Y. Tang, and X. K. Ouyang, "Shear-Induced Breakup of Cellulose Nanocrystal Aggregates," *Langmuir*, vol. **33**, no. 1, pp. 235–242, Jan. (2017).

[9] S. Ferrari and M. Serra, "An update on chemotherapy for osteosarcoma," *Expert Opin Pharmacother*, vol. **16**, no. 18, pp. 2727–2736, Dec. (2015).

[10] M. A. Harris and C. J. Hawkins, "Recent and Ongoing Research into Metastatic Osteosarcoma Treatments," *Int J Mol Sci*, vol. **23**, no. 7, Apr. (2022).

[11] S. C. Howard, J. McCormick, C.-H. Pui, R. K. Buddington, and R. D. Harvey, "Preventing and Managing Toxicities of High-Dose Methotrexate," *Oncologist*, vol. **21**, no. 12, pp. 1471–1482, Dec. (2016).

[12] E. Hendershot et al., "Outpatient High-dose Methotrexate for Osteosarcoma: It's Safe and Feasible, If You Want It," *J Pediatr Hematol Oncol*, vol. **41**, no. 5, pp. 394–398, Jul. (2019).

[13] A. A. Al-esnawy, K. T. Ereiba, A. M. Bakr, and A. S. Abdraboh, "Characterization and antibacterial activity of Streptomycin Sulfate loaded Bioglass/Chitosan beads for bone tissue engineering," *J Mol Struct*, vol. **1227**, (2021).

[14] A. E. Malash, A. A. Al-esnawy, K. T. Ereiba, A. M. Bakr, and A. S. Abdraboh, "An In Vitro Study of the Effects of Methotrexate Loaded Biocomposite Beads on MG63 Osteoblast Cells," *Sci Rep*, vol. **15**, no. 1, pp. 1–16, Dec. (2025).

[15] M. S. El-khooly, A. A. Abdel-Aal, A. E. Mekky, A. A. Al-esnawy, and A. S. Abdraboh, "'Tenoxicam-loaded bioglass/polyvinyl alcohol composites for biomedical applications: In-vitro study,' *Mater Chem Phys*, vol. **336**, p. 130526, May (2025).

[16] H. Ren, Y. Cui, A. Li, and D. Qiu, "Bioactive glass sol as a dual function additive for chitosan-alginate hybrid scaffold," *Chinese Chemical Letters*, vol. **29**, no. 3, pp. 395–398, Mar. (2018).

[17] A. Al-esnawy, A. Abd Raboh, A. Bakr, and K. Tohamy, "Bioactivity Study of Streptomycin Sulfate loaded Chitosan/Bioglass Scaffold.," *Egyptian Journal of Biomedical Engineering and Biophysics*, vol. **22**, no. 1, pp. 1–11, Mar. (2021).

[18] L. Fan, Y. Lu, L. Y. Yang, F. Huang, and X. kun Ouyang, "Fabrication of polyethylenimine-functionalized sodium alginate/cellulose nanocrystal/polyvinyl alcohol core–shell microspheres ((PVA/SA/CNC)@PEI) for diclofenac sodium adsorption," *J Colloid Interface Sci*, vol. **554**, pp. 48–58, Oct. (2019).

[19] T. Kokubo and H. Takadama, "How useful is SBF in predicting in vivo bone bioactivity?," *Biomaterials*, vol. **27**, no. 15, pp. 2907–2915, May (2006).

[20] O. Sánchez-Aguinagalde, A. Lejardi, E. Meaurio, R. Hernández, C. Mijangos, and J. R. Sarasua, "Novel Hydrogels of Chitosan and Poly(vinyl alcohol) Reinforced with Inorganic Particles of Bioactive Glass," *Polymers* 2021, Vol. **13**, Page 691, vol. 13, no. 5, p. 691, Feb. (2021).

تصميم وتقييم حوامل هجينة من الزجاج الحيوي/البوليمرات محمّلة بالميثوتركسات لهندسة أنسجة العظام

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تتناول هذه الدراسة تصنيع وتقييم حامل هجين جديد طور ليكون منصة واعدة لتوصيل الدواء بشكل موضعي ومستمر، على أن يتم تأكيد ذلك في دراسات الإطلاق المستقبلية. يدمج الحامل الزجاج الحيوي مع شبكة بوليمرية مكونة من بولي فينيل الكحول(PVA) ، وبلورات السليلوز النانوية(CNC) ، وألجينات الصوديوم .(SA) تم تحميل عقار الميثوتركسات (MTX) داخل مصفوفة PVA-CNC-SA المدعمة بكمية ثابتة (0.4 جم) من الزجاج الحيوي ($65\%\ SiO_2 - 30\%\ CaO - 5\%\ P_2O_5$) باستخدام تقنية التجفيف بالتجميد، مما نتج عنه تركيبة محسنة ذات بنية مسامية ونشاط حيوي مرتفع ملائم لتطبيقات هندسة أنسجة العظام.
تم توصيف الحامل الناتج بدقة باستخدام تقنيات FTIR و XRDو SEM وEDXA، كما تأكدت حيويته من خلال الغمر في سائل الجسم المحاكي (SBF)، حيث دل تكون طبقة هيدروكسي أباتيت على استجابة حيوية قوية. وعلى الرغم من عدم إجراء تجارب كمية لإطلاق الدواء في هذه المرحلة، فقد صمم النظام أساسا لتحقيق توصيل متحكم به لعقار الميثوتركسات.
بوجه عام، تظهر النتائج الإمكانات الواعدة للحامل الهجين المجفف بالتجميد كمنصة متعددة الوظائف قادرة على دعم تجديد العظام وتوفير توصيل موضعي للعلاج الكيميائي.