Journal of Mines, Metals and Fuels, *71(12A): 15-20; 2023. DOI: 10.18311/jmmf/2023/43176*

Print ISSN : 0022-2755

Journal of Mines, Metals and Fuels

Contents available at: *www.informaticsjournals.com/index.php/jmmf*

CoO Substituted Borate 1393B3 Glass Scaffold with Enhanced Metallurgical Performance

Md Ershad[1]*, Ranjan Kumar[1], Neeraj Gupta[2], Arijit Mukherjee[1], Soumya Ghosh[1] and Apurba Mandal[2]

[1]Department of Mechanical Engineering, Swami Vivekananda University, Barrackpore, Kolkata - 700121, West Bengal, India; mdershad.rs.cer13@iitbhu.ac.in

[2]Department of Mechanical Engineering, National Institute of Technology, Pauri - 246174, Uttarakhand, India

Abstract

In this study, we fabricated three-dimensional porous scaffolds using 1393B3 Borate-Based Glass (BBG) with a structure resembling trabecular bones. These scaffolds were created through a process involving melt-quenching and foam replica techniques. To evaluate the impact of incorporating CoO into these scaffolds on their biological compatibility, bioactivity, and physical-mechanical properties, we conducted a series of in vitro experiments. Our findings indicate that the CoO-infused scaffolds, referred to as CBBGs (CoO-derived 1393B3), exhibit superior mechanochemical stability compared to the original BBG scaffolds. Importantly, this enhancement in stability did not compromise the bioactivity or cytocompatibility of the scaffolds following CoO incorporation. In fact, our assessments of biological compatibility, including MTT assays, Live/Dead staining, and cell adhesion studies using L929 cell lines, revealed improved performance in the CBBGs, particularly in scaffolds containing up to 1% CoO (C1BBG and C2BBG). Notably, among the CBBGs, C1BBG consistently demonstrated the highest level of enhanced biological compatibility. In summary, our study demonstrates that the incorporation of CoO into BBG scaffolds enhances both their mechanical and biological performance without negatively affecting their bioactivity. Therefore, these CoO-infused BBG scaffolds have the potential to serve as innovative biomaterials for regenerating neo bone tissue.

Keywords: *Bioactivity, Bone-Tissue Engineering, Glass-Scaffold, Microhardness.*

1.0 Introduction

Glasses are renowned for their ability to resist crystallization during cooling from viscous melts. In borate glasses, B_2O_3 acts as the primary glass network former, akin to the role of SiO_2 in silicate glasses. Despite being network formers, they differ in viscosity as molten material and propensity to crystallize. Borate glasses demonstrate easier viscous flow behavior than silicate glasses at their liquidus temperature, rendering them more susceptible to devitrification. Bioactive glasses stand out from traditional glasses due to their distinct composition, properties, and applications. Coined by Professor Lary L. Hench in the late 1960s, the term "bioactive glasses" marked the beginning of a revolution in tissue engineering[1]. These glasses possess the unique ability to elicit specific responses from host tissue, facilitating a strong bond between material and tissue at the interface.

In the case of 1393B3 glasses, they depart from conventional glasses as B_2O_3 completely replaces SiO_2 as the primary glass network former. The typical chemical composition includes B_2O_3 (56.6%), CaO (18.5%), Na_2O (5.5%), MgO (4.6%), K_2O (11.1%), and P_2O_5 (3.7%) by weight. Upon sintering, 1393B3 glass follows kinetics

*Author for correspondence

similar to 45S5, transforming into glass-ceramics. Controlled heat treatment is necessary to form glass fibers or scaffolds from borate-based bioactive glasses[2,3].

Due to their faster degradation and subsequent conversion to bone minerals such as HA or $Ca_{10}(PO_4)_6(OH)_2$, borate-based bioactive glasses are preferred for tissue engineering applications. They convert to bone minerals more rapidly and efficiently than silicate glasses, without leaving behind a silica-rich layer. Additionally, the conversion of HA is believed to be more controllable in borate glasses compared to silicate glasses[4,5].

Boron, an essential trace element, plays a crucial role in maintaining healthy bones and possesses therapeutic properties beneficial for bone and wound healing. Its presence in bioactive glasses introduces therapeutic benefits such as osteogenesis and angiogenesis. Cobalt has also been studied as an additive in bioactive glass to enhance mechanical properties, wound healing, and neovascularization. However, there are toxicity limits for cobalt in bioactive glass, with studies suggesting permissible levels between 0.8% to 3.0% by weight. Studies have shown insignificant cytotoxicity and maximum cellular viability up to 2.0% CoO incorporation into 1393 glass scaffolds[6,7].

2.0 Materials and Methods

2.1 Scaffolds and SBF Preparation

The glass materials were melted at 1350±10 °C using crucibles. The batches were prepared by blending precise quantities of raw materials in agate mortars for 30 minutes. Raw materials included AR grade of oxides. After melting, the glass melts were quenched in double-distilled water, dried, crushed, and ground. The resulting glass powder underwent ball milling in ethanol with Al_2O_3 balls for 2-4 hours. A slurry swas prepared by mixing the powder with water and ethanol, then adding a 5.0 wt % PVA solution. PU foams were immersed in the slurry, ensuring even coating, then dried at 80˚C for 30 minutes. Excess slurry was removed, and foams were dried at 120˚C for two days before sintering at 700-750 ˚C to form glass-ceramic scaffolds for both BBG and CBBGs.

The preparation of SBF followed the protocol outlined by Kokubo *et al*[8]. The detailed procedure has been documented elsewhere. In brief, SBF preparation involves sequentially adding (NaCl), ($MgCl_2.6H_2O$), (KCl), ($CaCl_2$), $K_2HPO_4.3H_2O$, $NaHCO_3$, $Na2SO_4$, and 1 M (HCl) to 1000 ml of Double-Distilled (DD) water. Each reagent is added individually, ensuring complete dissolution. The resulting solution is then buffered to a pH of 7.4 at 37°C using Tris (tris-hydroxymethyl aminomethane; $C_4H_{11}NO_3$) and stored at 4°C. This prepared SBF solution serves as the medium for evaluating the Bioactivity of Borate Glasses (BBGs).

2.2 Methods

The in vitro bioactivity of BBGs was evaluated by immersing the samples in SBF for predetermined durations. Assessment methods included X-ray Diffraction (XRD), Fourier-transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy with Energy Dispersive X-ray Analysis (SEM-EDX), and monitoring the pH of the solution.

2.2.1 XRD

XRD analysis of the glass samples was conducted using a

Table 1. Chemical composition of 1393B3 glass in mole%

Code (Scaffolds)	B_2O_3	CaO	Na_2O	K_2O	MgO	P_2O_5	CoO
BBG (1393B3)	54.6	22.1	6	7.9	6.7	2.7	0
C1BBG (1393B3-0.5 % CoO)	54.1	22.1	6	7.9	6.7	2.7	0.5
C2BBG (1393B3-1.0 % CoO)	53.6	22.1	6	7.9	6.7	2.7	1
C3BBG (1393B3-2.0 % CoO)	52.6	22.1	6	7.9	6.7	2.7	2

Rigaku Miniflex II X-ray diffractometer from Japan. The instrument featured a graphite monochromatized Cu-Kα X-ray source and a Ni filter, operating at a tube voltage of 40 kV and a current of 20 mA. XRD scans were performed within an angular range (2θ) of 10-80˚, with a scan rate set at 3˚ per minute. The obtained data were analyzed using PANalytical's X'Pert High Score Plus software, matching them with standard ICDD (International Centre for Diffraction Data) reference cards.

2.2.2 FTIR

For identifying functional groups responsible for new peaks and characterizing existing peaks in the glass samples, an FTIR (Fourier Transform Infrared) spectrometer from BRUKER, the Tensor 27 model from Germany, was employed. FTIR analysis included acquiring 32 scans at a resolution of 4 cm^{-1} in transmittance mode, spanning the wavenumber range of 4000 to 400 cm^{-1}. This facilitated a detailed examination of spectral features associated with the glass samples.

2.2.3 SEM-EDAX

The morphological examination of the glass scaffolds (both BBG and CBBGs) soaked in SBF was conducted using Scanning Electron Microscopy (SEM). An EVO|18 smart SEM instrument from ZEISS in the United States equipped with an Energy Dispersive X-ray Analysis (EDAX) system was utilized for this analysis. The SEM operated at an accelerating voltage of 20 keV (EHT).

The primary objective of this examination was to analyze the surface microstructure and morphology of the samples treated with SBF, focusing particularly on observing the development of layers resembling Hydroxyapatite (HA).

2.3 Evaluation of Mechanochemical Performance of Porous BBGs

The mechanical properties of the scaffolds were evaluated using a Universal Testing Machine (UTM) H10KL from Tinius Olsen in the United States. Testing conditions included maintaining a crosshead speed of 0.05 mm/min and utilizing a 10 kN load cell. To assess the mechanical stability of the samples in a physiological liquid environment, compression and three-point bending tests were conducted on samples with specified dimensions, both in dry (sintered only) and wet conditions (after 15 days of soaking).

The flexural strengths of the porous glass scaffolds were determined following the standardized ASTM C1674-11 protocol. Additionally, the bending modulus was calculated based on the stress-strain relationship. Detailed computations for these mechanical properties were performed accordingly.

$$\text{Compressive stress, } \sigma_c = \frac{P}{A}$$

$$\text{Flexural stress, } \sigma_f = \frac{3PL}{2BD^2}$$

$$\text{Strain, } \varepsilon = \frac{6Dd}{L^2}$$

$$\text{Modulus of flexure, } E_f = \frac{L^3 m}{4BD^3}$$

In the equations provided: \(P \) represents the load in Newtons, \(L \) stands for the outer span length in millimeters, \(A \) denotes the surface area of the load in square millimeters, \(D \) represents the depth of the scaffolds in millimeters, \(B \) signifies the breadth of the scaffolds in millimeters, \(d \) indicates the deflection or midspan displacement in millimeters, and \(m \) represents the gradient of the initial straight-line portion of the load-deflection curve in N/mm..

3.0 Results

3.1 *In vitro* Bioactivity Evaluation through Structural, Functional, Morphological, and Physico-Chemical Analysis

BBG and CBBGs underwent bioactivity assessment following immersion in Simulated Body Fluid (SBF) for 15 days. X-ray diffractogram data showed significant changes in characteristic peaks between the soaked and sintered-only samples. Pronounced peaks, particularly at angular values of 32˚, 45˚, and 56˚, were observed in the SBF-treated scaffolds, indicating the formation of hydroxyapatite (HA). Interestingly, peak intensities in CBBGs were enhanced in the SBF-treated samples, with

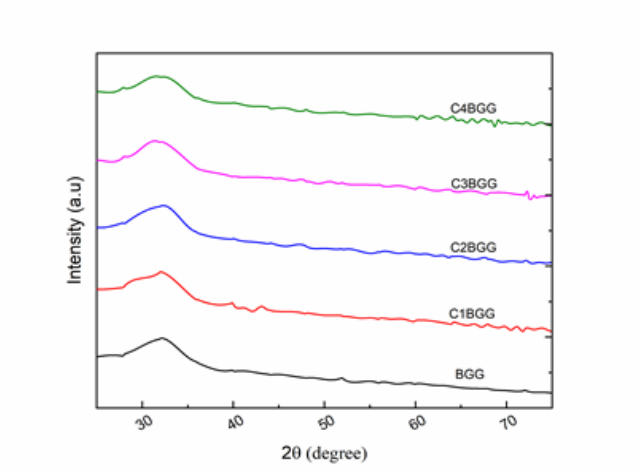

Figure 1. XRD of BBGs before 'soaking in SBF.

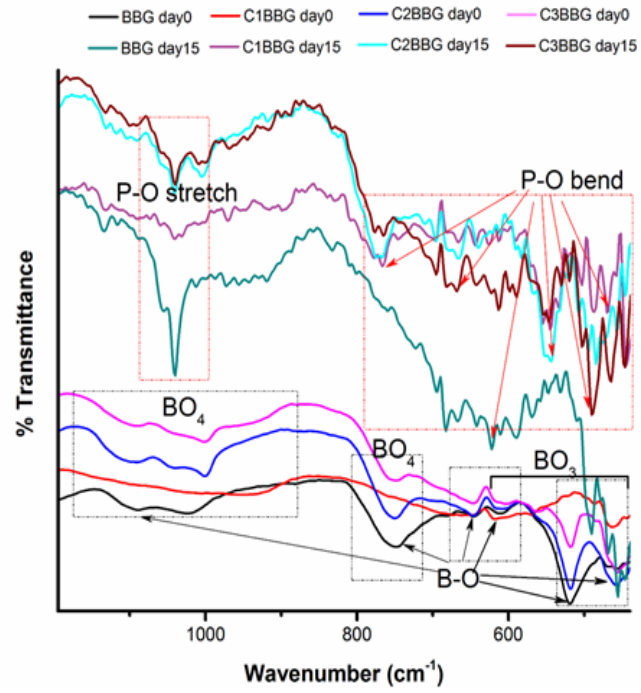

Figure 2. FTIR of BBGs showing characteristics resonances of HA formation due to soaking in SBF (above) comparing the untreated samples (below).

the enhancement correlating with the percentage of CoO in the composition of 1393B3.

FTIR spectra analysis was conducted to identify molecular functional groups in the glass samples. Bending vibrations were observed in the range of 400-700 cm^{-1}. Significant shifts in resonant frequencies were observed within the range of 400-1200 cm^{-1} after immersion in Simulated Body Fluid (SBF) for 15 days. In the sintered-only samples, prominent characteristic bands were observed at specific frequencies. However, soaked samples showed new vibrational resonances at different frequencies. Additionally, higher-frequency vibrational bands corresponding to OH groups were detected in the range of 2800-3700 cm^{-1}.

In addition to structural characterization, functional and chemical analyses, along with morphological assessments, are crucial for determining the in vitro bioactivity of bioactive glass samples. Figure 3 presents morphological micrographs of BBGs obtained from SEM-EDX analysis.

The microscopic images reveal needle/rod-like nonhomogeneous phases over the BBG and C1BBGs. However, on C2BBG and C3BBG, newly developed phases appear more like clusters of granules or elongated particles rather than rod-like morphologies. EDX analysis confirms the presence of elemental calcium and phosphate, along with other elements, except for boron, which was not detected due to instrument limitations.

4.0 Discussion

X-ray diffractometry was utilized to analyze the bioactivity of our materials by examining crystallographic changes after immersion in Simulated Body Fluid (SBF). The appearance of pronounced peaks corresponding to hydroxyapatite (HA) crystals at specific angles confirmed the bioactive nature of our scaffolds (BBG and CBBGs). Interestingly, the addition of cobalt (CoO) did not compromise the bioactivity of the materials; instead, CBBGs showed enhanced bioactivity compared to BBG. SEM micrographs revealed the presence of needle or rod-like nonhomogeneous phases and dispersed hydroxyapatite particles on the glass surfaces, corroborated by the presence of elemental phosphate and calcium in the EDX spectrum, indicating apatite layer formation. The calculated Ca/P ratio further supported the similarity of the samples to bone composition.

Physical analysis indicated a high porosity (51-56 %) with pore sizes predominantly greater than 100 μm. Mechanical characterization demonstrated significant improvements in compression, flexural strength, elastic modulus, and toughness modulus of CBBGs compared to BBG, attributed to CoO incorporation. The addition

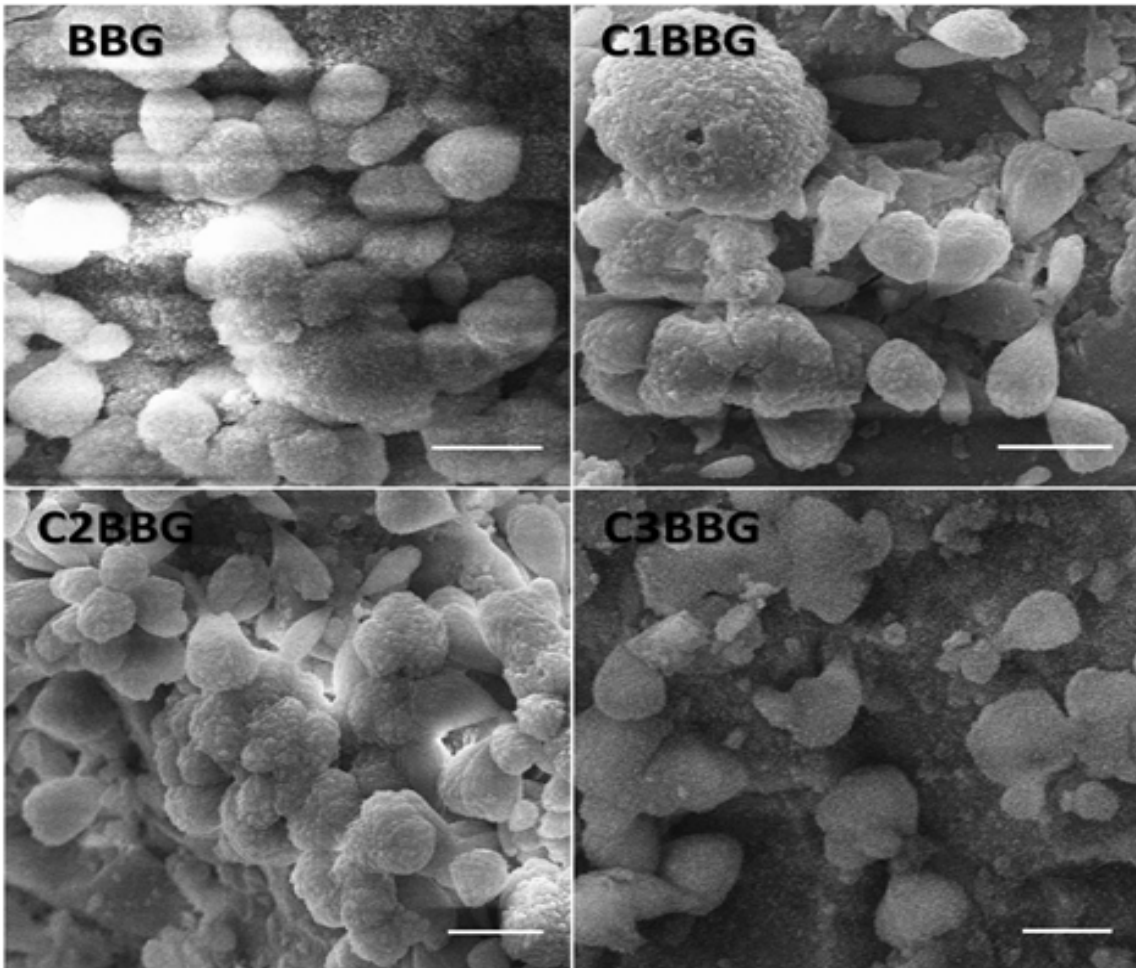

Figure 3. SEM and EDAX analysis of samples assessing surface modification due to HA formation. Inside bar=10 µm.

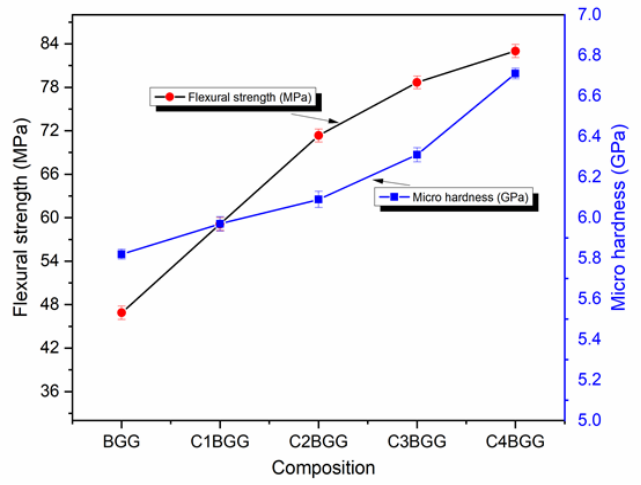

Figure 4. Mechanical properties: Compressive and flexural strengths of the BBGs were found enhanced post CoO incorporation.

of CoO enhanced the elastic properties of the scaffolds, reduced brittleness, and increased toughness, resulting in a more linear stress-strain profile.

Overall, the incorporation of metallic properties through CoO addition reinforced the glass structure, enhancing mechanical performance without compromising bioactivity, making the CBBGs promising candidates for various tissue regenerative applications.

5.0 Conclusion

In this study, we fabricated 3D porous scaffolds using 1393B3 bioactive glass (BBG) and its CoO derivatives (CBBGs) via foam replication. These scaffolds were designed to replicate the structure of cancellous bone, consisting of interconnected porous struts. We conducted

analyses to assess their in vitro biological performance and mechanochemical reliability.

Our findings from the in vitro assessments indicate that the scaffold incorporating 0.5% CoO exhibited optimal mechanical strength and improved biological compatibility. This suggests that the inclusion of CoO enhances both the mechanical properties and biocompatibility of the scaffolds. Additionally, we anticipate that the enhanced mechanical properties of CBBGs, attributed to CoO incorporation, will improve their ability to withstand physiological loads.

Furthermore, our observations revealed a controlled release of ions from the CBBGs, leading to a gradual conversion of the glass material into hydroxyapatite (HA). This controlled ion release mechanism is crucial for facilitating bone regeneration processes.

Overall, the CBBGs demonstrate a combination of desirable qualities essential for bone tissue regeneration applications. Considering their enhanced mechanical performance, biological compatibility, and controlled ion release behavior, CBBGs show promise for use in bone tissue engineering and regenerative medicine.

6.0 References

1. Hench LL, Xynos ID, Polak JM. Bioactive glasses for *in situ* tissue regeneration. Journal of Biomaterials Science. Polymer Edition. 2004; 15(4):543-62. https://doi.org/10.1163/156856204323005352 PMid:15212333
2. Schmelzer J, Röpke G, Priezzhev VB. Nucleation theory and applications. Wiley-VCH Weinheim. 2005. https://doi.org/10.1002/3527604790
3. Rahaman MN, Day DE, Bal BS, Fu Q, Jung SB, Bonewald LF, Tomsia AP. Bioactive glass in tissue engineering. Acta Biomaterialia. 2011; 7:2355-73. https://doi.org/10.1016/j.actbio.2011.03.016 PMid:21421084 PMCid:PMC3085647
4. Xia L, Ma W, Zhou Y, Gui Z, Yao A, Wang D, Takemura A, Uemura M, Lin K, Xu Y. Stimulatory effects of boron-containing bioactive glass on osteogenesis and angiogenesis of polycaprolactone: *in vitro* study. BioMed Research International. 2019. https://doi.org/10.1155/2019/8961409 PMid:31011582 PMCid:PMC6442456
5. Ali A, Ershad M, Hira S, Pyare R, Singh SP. Mechanochemical and *in vitro* cytocompatibility evaluation of zirconia-modified silver-substituted 1393 bioactive glasses. Boletín de la Sociedad Española de Cerámica y Vidrio. 2020.
6. Ali A, Ershad M, Vyas VK, Hira SK, Manna PP, Singh B, Yadav S, Srivastava P, Singh S, Pyare R. Studies on the effect of CuO addition on mechanical properties and *in vitro* cytocompatibility in 1393 bioactive glass scaffold. Materials Science and Engineering: C. 2018; 93:341-55. https://doi.org/10.1016/j.msec.2018.08.003 PMid:30274066
7. Ali A, Singh BN, Hira SK, Singh S, Pyare R. ZnO modified 1393 bioactive scaffolds with enhanced cytocompatibility and mechanical performance. Ceramics International. 2020; 46:6703-13. https://doi.org/10.1016/j.ceramint.2019.11.159
8. Kokubo T, Takadama H. How useful is SBF in predicting *in vivo* bone bioactivity? Biomaterials. 2006; 27:2907-15. https://doi.org/10.1016/j.biomaterials.2006.01.017 PMid:16448693