

# Engineering Restorative Scaffolds for Hyperglycemia-Targeted Bone Healing in Diabetes Mellitus

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**INTRODUCTION:** Treating bone defects in diabetes mellitus (DM) patients with higher fracture risks and impaired healing, remains an unmet clinical need. Biocompatible polymer scaffolds show promise for guiding bone regeneration. Current DM fracture scaffolds, using conventional materials and glycemic control drugs, often fail due to advanced glycation end-products (AGE) accumulation, affecting scaffold-native tissue integration. By addressing the effects of hyperglycemia and AGEs on bone tissue, the proposed precision bone restorative scaffold and localized treatments offer a promising approach to enhance bone healing in diabetic individuals. We designed a multifunctional scaffold that prevents AGE buildup, supports osteoblastogenesis, and enhances bone healing. We hypothesize that delivering glucose oxidase (GOx) enzyme can hinder AGE formation and promote osteoblastogenesis while preventing collagen matrix degradation due to non-enzymatic crosslinking (**Fig 1a**).

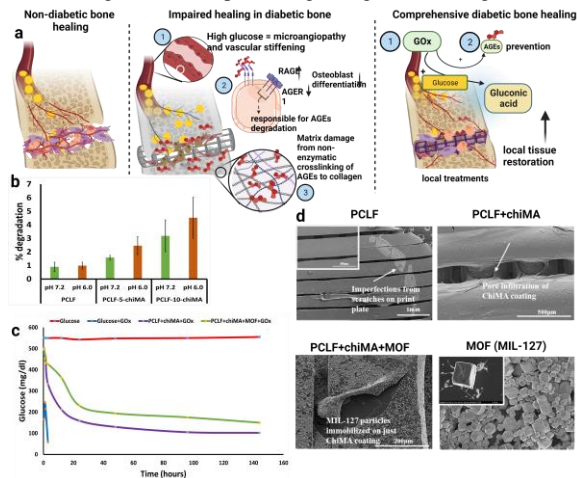


Figure 1: Schematic representation of research concept and material characterization

additional chiMA layers were applied (**Fig 1d**). Coating degradation measurement for 5 and 10 layers of chiMA over 7 days exhibited lower degradation of 5-layer chiMA coating in both neutral (7.2) and acidic (6.0) pH conditions (**Fig 1b**). We observed sustained GOx release over 144h with gradual glucose reduction from 550mg/dl to as low as 105mg/dl. Both PCLF+chiMA+GOx and PCLF+chiMA+MOF+GOx scaffolds were successful in lowering the amount of glucose to normoglycemic levels. Moreover, the presence of MIL-127 extended the reaction life of the enzyme and served as a potential vehicle for further functionalization of these scaffolds (**Fig 1c**). HG cultured OBs at 7 and 14 days exhibited reduced RUNX2 and increased RAGE expression, indicating AGE-impaired differentiation. Shifting to NG conditions restored RUNX2 and ALP (**Fig 2b**). This finding was supported by decreased alkaline phosphatase (ALP) production in OBs exposed to HG over 14 days (**Fig 2c**). Confocal micrographs confirmed HG conditions did not result in cell death but had significant contribution towards delayed proliferation of OBs. Therefore for *in vitro* studies with GOx releasing scaffolds, OB morphology and proliferation had been studied. The formation of H<sub>2</sub>O<sub>2</sub> in the culture media as a function of GOx-glucose reaction has apoptotic effects on cells in general. Consistent with that, we also observed abnormal cellular behavior due to H<sub>2</sub>O<sub>2</sub> formation. To mitigate such an anomaly, NaPY was incorporated into the sustained release chiMA coating to act as a decarboxylation agent to reduce the amount H<sub>2</sub>O<sub>2</sub> in the media during culture. OBs cultured with NaPY+GOx-releasing scaffolds exhibited survival, early restoration of cellular morphology and proliferation at day 2, confirming scaffold efficacy in lowering glucose levels towards restored OB functions in hyperglycemia (**Fig 2a**).

**DISCUSSION:** Our results show a synergized enhancement of osteoblast differentiation in hyperglycemic conditions possible via reversing the high-glucose conditions through enzymatic interactions. We have successfully established the effect of such a multifunctional scaffold-facilitated reaction on diabetes related glucose metabolic markers toward osteoblast differentiation. We have observed that reducing glucose-levels in cellular microenvironment has a significant impact on AGE receptors (RAGE) which is primarily responsible for cellular non-functioning due to hyperglycemia. Our coating is multifunctional which can also be translated to other biomaterials as well as biomedical devices. The contribution of AGE reduction towards reversal of hyperglycemic damage to bone microenvironment via tailored biomaterial is novel and has the potential to exploring scaffold customization based on comorbidities of patients. Our future studies will involve investigating glucose reduction in congruence with local insulin delivery towards restoring cellular functions in DM bone via *in vivo* studies. We will also deliver biologics to eliminate AGE produced in the bone thereby comprehensively prevent as well as treat AGE related bone healing disruptions.

**SIGNIFICANCE:** Our innovative multifunctional precision scaffold offers a solution to the challenges of diabetic bone healing. By preventing AGE accumulation within the bone matrix, a common hindrance to successful healing in DM, and incorporating glucose oxidase (GOx) to preserve OB differentiation, our approach is tailored to address the specific metabolic dysregulation in DM. This research holds great promise for advancing diabetic bone healing and meeting the unmet clinical need for effective DM treatments.

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**METHODS:** Biocompatible and biodegradable poly(caprolactone fumarate) (PCLF) scaffolds with 1 mm pores were 3D printed, cleaned, and coated in 5 layers of 2% chitosan methacrylate (chiMA) containing a photo crosslinker and 20 mM sodium pyruvate (NaPY) using UV crosslinking. MIL-127 metal organic frameworks (MOF) loaded with 0.02 mg/ml GOx were synthesized for sustained glucose reduction in cell culture media over 7 days. *In vitro* studies included osteoblast (OB) cultures on polystyrene plates with treatment scaffolds contained in transwell inserts under normal glucose (NG) and high glucose (HG) conditions. OB morphology and differentiation were assessed through immunostaining, qPCR, and hydroxyproline-fAGE assay for collagen crosslinking. GOx-releasing scaffolds were tested for their impact on hyperglycemic OB differentiation. MIL-127 was functionalized and characterized using Field Emission Scanning Electron Microscopy (FESEM). Coating degradation was assessed in pH 6.0 and 7.2 buffers over 7 days. Controlled release of GOx from MIL-127 immobilized chiMA coating was studied for various GOx-to-glucose ratios. *In vitro* cell culture involved two sets of experiments: one observing OB viability and differentiation under NG and HG conditions, and the other using GOx-releasing scaffolds. Statistical analyses utilized one-way ANOVA with Tukey-Kramer's correction at 95% confidence.

**RESULTS:** FESEM micrographs show successful chiMA coating with a uniform surface morphology. It also confirmed the immobilization of synthesized MIL-127 to the chiMA coating, with minimal leaching observed when

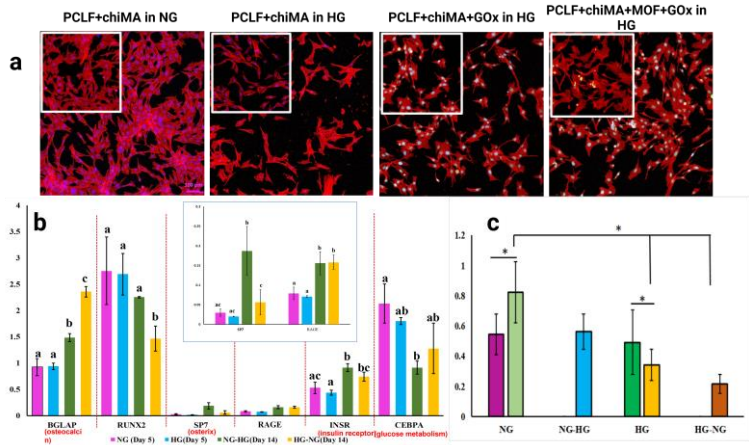


Figure 2: In vitro osteoblast proliferation and differentiation for GOx releasing scaffolds