

Oxygen-Releasing Calcium Peroxide nanoparticles embedded in hydrogels as a strategy for overcoming Hypoxia in large 3D tissue constructs

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Word count: 1500

Keywords: nanoparticles, hydrogels, hypoxia, tissue engineering, bioscaffolds

Funding: The author received no specific funding for this work.

Conflict of interest: The author declares no conflict of interest.

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Introduction

Hypoxia remains a major barrier to engineering large and complex 3D tissue constructs, as oxygen diffusion limits restricts cell survival in thick scaffolds [1,2]. Hypoxic environments are particularly problematic in chronic wounds and tissue defects, where limited oxygen availability delays healing and contributes to infection and inflammation [3]. Addressing oxygen deprivation is essential to improve tissue viability and regenerative tissue outcomes. Within the last 20 years, calcium peroxide (CaO_2) nanoparticles have emerged as effective oxygen-releasing materials (ORMs), capable of sustained oxygen delivery through hydrogen peroxide (H_2O_2) decomposition within biocompatible hydrogel matrices [4–6]. These nanomaterials have been successfully embedded to enhance cellular vascularization in preclinical models [4,7]. Despite promising *in vivo* studies and animal data, translating CaO_2 -based oxygen-releasing biomaterials to clinical applications encounters persistent challenges such as uncontrolled oxygen release and the risk of generating H_2O_2 [8]. This viewpoint reviews novel advances in CaO_2 oxygen-releasing polymers while evaluating efforts to translate these systems into clinical application through scaffold engineering. Consideration of a variety of limitations is examined, emphasizing why targeted research is paramount to refining these engineered systems for therapeutic use.

Effects of Hypoxia in Engineered Tissue Systems

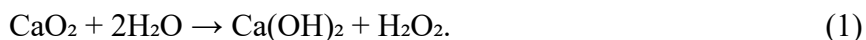
A scaffold is an artificial 3D structure designed to support the growth of new cellular matter. Oxygen can only diffuse approximately 100-200 micrometers (μm) into interconnected tissue pores before its concentration falls sharply, creating steep oxygen gradients that result in severe hypoxic or anoxic conditions in the scaffold “core” of the fiber construct [1,2,9]. This steep gradient arises because oxygen has low solubility and diffusivity in aqueous environments [10], and the high metabolic rates of embedded cells rapidly consume available oxygen. Matrix density influences this diffusion limit, with denser matrices impeding oxygen transport and high cell densities accelerating oxygen depletion [10,12]. These limitations are particularly detrimental in cardiac tissue engineering, where cardiomyocytes require continuous oxygen supply for contractile activity, in bone regeneration that demands oxygen for osteogenic differentiation, and in skin or wound healing where oxygen scarcity delays re-epithelialization and weakens immune defense mechanisms [3,11].

Under normal oxygen levels, hypoxia-inducible factors (HIF) alpha subunits are hydroxylated by prolyl hydroxylase domain (PHD) proteins, which target them for degradation via the proteasome. However, under hypoxic conditions, PHD activity is inhibited, leading to the stabilization and accumulation of HIFs. These transcription factors mediate adaptation by shifting metabolism toward anaerobic glycolysis and promoting angiogenesis through the secretion of vascular endothelial growth factor (VEGF), which stimulates the formation of new blood vessels [4]. While HIF activation initially supports cell survival under low oxygen, prolonged hypoxia can lead to necrosis and delayed wound healing [1,4]. Conventional methods like perfusion bioreactors improve oxygen distribution by circulating oxygenated media but are typically unsuccessful in oxygenating scaffold interiors effectively, and pre-vascularization approaches fail in functional integration with host vasculature (e.g., existing blood vessels) following implantation [8,13]. To overcome these challenges, CaO_2 -based oxygen-releasing

materials have been incorporated into scaffolds to provide ongoing, localized oxygen release [4-7,14].

CaO₂ Oxygen Release

CaO₂ is a solid inorganic peroxygen compound commonly used in oxygen-releasing biomaterials due to its ability to gradually release oxygen when in contact with water [1,5]. It decomposes in aqueous environments through hydrolysis, releasing oxygen via a two-step reaction. First, CaO₂ reacts with water to form calcium hydroxide and H₂O₂ (Equation #1):



Then, the H₂O₂ decomposes into water and molecular oxygen (Equation #2), a process frequently catalyzed by endogenous enzymes like catalase to reduce oxidative stress:



This sequential reaction enables a biphasic burst, which is an initial release of surface-accessible, reactive oxygen species (ROS) produced by mitochondrial complex III and L-2-hydroxyglutaric acid (L-2HG), followed by sustained oxygen generation. [4,14-15]. The transient elevation of ROS can initiate angiogenic signaling, however; the accumulation can manifest hyperoxia-related peroxide cytotoxicity, especially in stem cells and densely vascularized provisional tissue composed of fibroblasts [10,15]. To mitigate these effects, CaO₂ is often encapsulated in protective matrices or coated with PEGylated shells, polycaprolactone, tannic-acid-modified alginate [4, 16-17]. Unlike other oxygen carriers, such as perfluorocarbons, which require pre-saturation with molecular oxygen to dissolve large amounts of gas, or magnesium peroxide, which hydrolyzes slowly and lacks the rapid initial burst, CaO₂ offers spontaneous, high-yield oxygenation with sufficient storage stability [1,6,18-19]. Tomioka et al. evaluated oxygen release from 1 mg mL⁻¹ CaO₂ in NaHCO₃ solutions with NaH₂PO₄, measuring dissolved oxygen (DO) where 100% equals atmospheric equilibrium. Below 0.01 mg mL⁻¹ NaH₂PO₄, CaO₂ showed burst release, reaching ~180% DO after 30 min, likely because amorphous calcium carbonate or CaCO₃ crystals did not form on the CaO₂ surface during dissolution–reprecipitation.

Additionally, CaO₂ has demonstrated incredible versatility across hydrogel networks and porous scaffolds used in regenerative medicine [1,5,20]. In chitosan-polyvinyl alcohol (PVA) composites, integrated particles release oxygen steadily over 3-5 days and enhance fibroblast migration and neovascularization in diabetic wound models [7,20]. Furthermore, tannylated alginate scaffolds incorporating CaO₂ have shown antibacterial efficacy against *S. aureus* due to ROS-mediated stress while maintaining oxygenation sufficient to support cell survival under hypoxic culture [4,21-22].

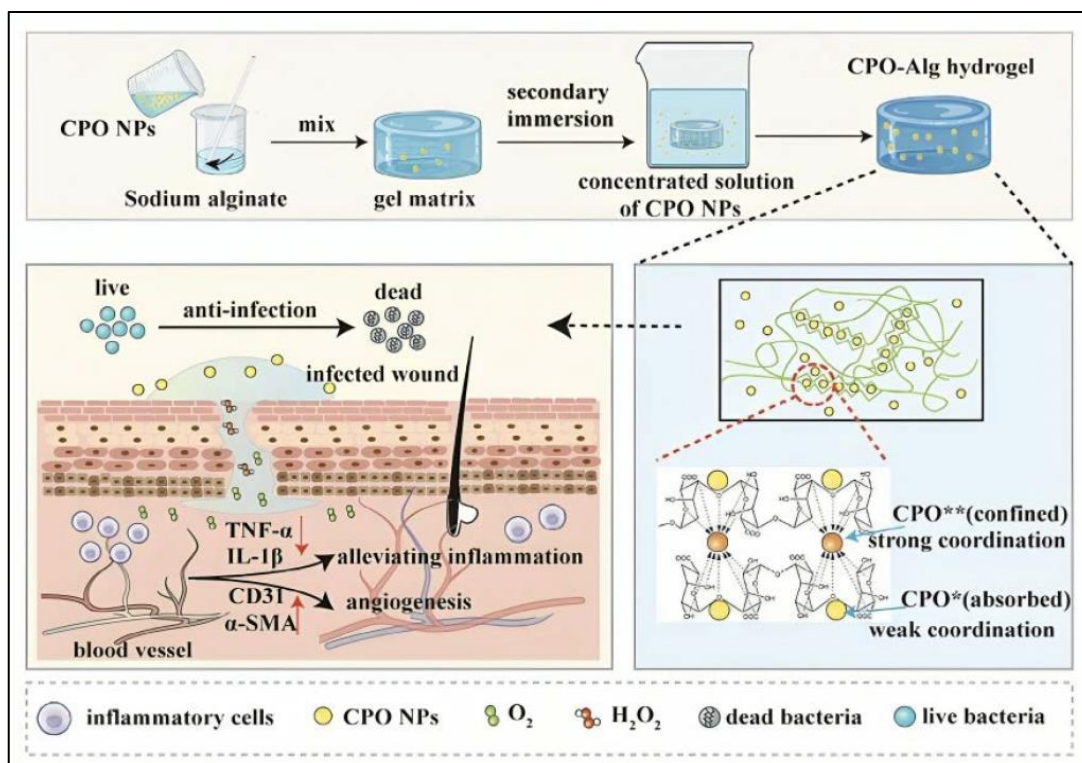


Figure 1. Schematic diagram of the preparation of CPO-Alg hydrogel and their application for infected wound healing. CPO-Alg hydrogels were prepared through a two-step process. The two-step fabrication of CPO-Alg hydrogels facilitates the coexistence of CPO NPs with two distinct coordination states in the gel structure, allowing a tapered release of H_2O_2 . Scheme obtained from Huang et al. [5].

Hydrogel Matrices for Nanoparticle Embedding

Hydrogel matrices are 3D, water-rich polymeric networks that resemble the biochemical and mechanical characteristics of the extracellular matrix (ECM), enabling their widespread use in localized therapeutic transport, including oxygen-releasing systems [2,6,11]. As outlined above, hydrogel matrices are widely employed as carriers for CaO_2 due to their compatibility with biological tissues. Their hydrated polymer networks supply porous structures that support oxygen diffusion and secure localization of ROS particles, which is critical for targeted therapy in 3D tissue regeneration [2,9]. Choi et al. engineered a poly(lactic-co-glycolic acid) (PLGA) microsphere that continuously releases oxygen ($1\text{--}3\text{ mg L}^{-1}$) and incorporated it into a sponge-based hydrogel dressing called ‘oxygen-releasing hydrogel sponge’, which enhanced oxygen supply and induced neovascularization [23].

The pseudoplastic flowing behavior of many hydrogel formulations facilitates the homogeneous dispersion of solid nanoparticles through simple blending methods, in addition to supporting advanced embedding strategies such as coaxial bioprinting and microsphere encapsulation. [6,9,13]. Moreover, these techniques allow hydrogels to deliver oxygen in a temporally controlled, gradual manner and simultaneously shield the reactive particles from premature decomposition [24]. In other words, a sudden burst of oxygen or H_2O_2 , which could lead to an overly alkaline (extremely basic) environment, is avoided.

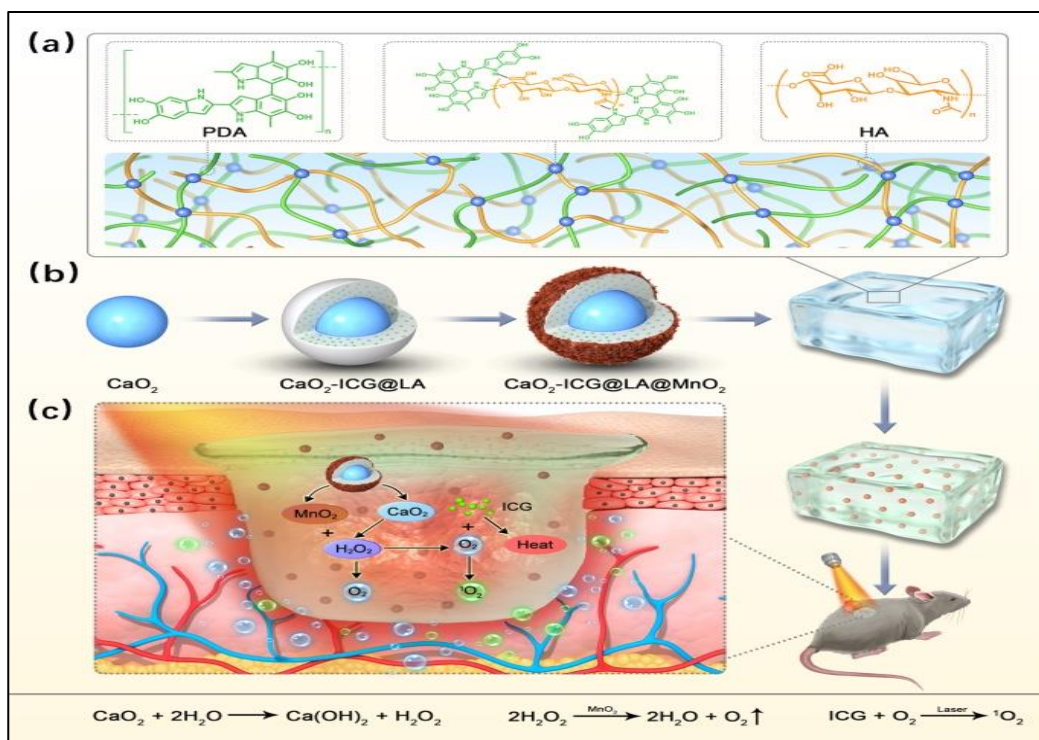


Figure 2. Photothermal/photodynamic, oxygen-generating CaO_2 microcapsules in a PDA/HA hydrogel for infection control and hypoxia relief. **(a)** Composition of the adhesive hydrogel matrix formed from polydopamine (PDA) and hyaluronic acid (HA). The interpenetrating network (blue cross-links) provides mechanical support and sites to immobilize catalytic microcapsules. **(b)** Stepwise construction of core-shell microparticles and their incorporation into the gel. A CaO_2 core is first wrapped with an ICG@LA layer to yield $\text{CaO}_2\text{-ICG@LA}$, then coated with MnO_2 to produce $\text{CaO}_2\text{-ICG@LA@MnO}_2$. **(c)** Therapeutic mechanism in infected wounds. Wound fluid triggers CaO_2 hydrolysis to Ca(OH)_2 and H_2O_2 ; the MnO_2 shell catalytically decomposes H_2O_2 into O_2 , limiting peroxide accumulation while enriching dissolved oxygen in the tissue. Near-infrared irradiation activates ICG to generate heat and singlet oxygen ($^1\text{O}_2$), enabling synergistic photothermal/photodynamic antibacterial activity. Reaction scheme: $\text{CaO}_2 + 2\text{H}_2\text{O} \rightarrow \text{Ca(OH)}_2 + \text{H}_2\text{O}_2$; $2\text{H}_2\text{O}_2 \xrightarrow{\text{MnO}_2} 2\text{H}_2\text{O} + \text{O}_2 \uparrow$; $\text{ICG} + \text{O}_2 \xrightarrow{\text{Laser}} ^1\text{O}_2$. Figure obtained from Li et al. [3].

Bioscaffold Engineering

Bioscaffolds are ECM mimics that offer spatially structured environments that support transplanted tissues and address post-implantation challenges like hypoxia, as seen in islet transplantation models [25]. They are generally classified by structural design into cryogels, nanofibrous networks, 3D-printed lattices, and injectable matrices. Each class offers a distinct mechanical profile suited for specific implantation contexts. Cryogels are a type of 3D porous polymer network formed by cryotropic gelation, meaning gelation occurs at sub-zero temperatures. They exhibit unique macroporous, sponge-like structures with enhanced non-linear elasticity, enabling rapid fluid absorption and high oxygen permeability [26]. Lattice structures are porous, interconnected support structures with a repeating pattern of bioink-based cells or voids. These structures can be classified as either 'acellular scaffolds'—those *without* cells, such as hip and knee implants or cranial implants—and 'cellular scaffolds,' which incorporate living

cells, as seen in tissue-engineered skin constructs [27]. Electrospun nanofibrous scaffolds provide a similar architecture that mimics the fibrous structure of native ECM, facilitating efficient oxygen diffusion and supporting directional tissue maturation [10]. Injectable oxygen-releasing scaffolds offer minimally invasive delivery and *in situ* conformation to irregular defect sites (in comparison to surgery), while permitting tunable, localized oxygen release [15,25].

Sustaining oxygenation in large tissue constructs requires scaffold designs that promote porosity and interconnectivity, achieved through via freezing rate manipulation in cryogels or nozzle displacement in extrusion-based bioprinting. This creates directional channels that facilitate oxygen diffusion and nutrient flow [9,27]. Adjusting pore sizes also reduces tortuosity, and improves permeability, allowing convective oxygen transport to central regions. Then, CaO_2 is integrated through either emulsion-based microencapsulation or coaxial extrusion, forming localized oxygen depots that preserve matrix uniformity [5,16]. To prevent premature degradation, surface modifications such as PEG-coating are applied, especially in shear-sensitive injectable platforms [1,18].

Conclusion and Clinical Translation

For CaO_2 nanoparticle–hydrogel systems to progress from laboratory studies to clinical therapies, future research must establish comprehensive means for regulating oxygen and H_2O_2 release in biologically safe ranges. This remains critical to avoid oxidative stress, which can impair healing or damage surrounding tissues if oxygen delivery rates exceed local demand [1,5,22]. To address this, future studies need to optimize particle microstructure and hydrogel crosslinking to regulate release kinetics for tissue-site-specific applications, ultimately providing more effective treatments. Additionally, the materials used in hydrogel-nanoparticle composites must be biocompatible with sterilization practices such as gamma irradiation or ethylene oxide exposure, without altering important mechanical properties or the way oxygen releases [26]. Furthermore, shelf-stable formulations that retain reactivity without aggregation or H_2O_2 -resulting degradation will be essential for real-world deployment in hospitals or operating rooms to avoid inducing cytotoxicity or apoptosis [29]. To achieve this, reproducible manufacturing protocols must be defined under good manufacturing practices (GMP) conditions to satisfy regulatory expectations for batch consistency, especially for complex composite systems [27].

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