Antimicrobial Oxygen-Generating Injectable Scaffold for Wound Healing

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Statement of Purpose:

Because of the limited diffusion properties of Three-Dimensional scaffold, oxygen deprivation is one of the challenges in the tissue engineering field. Delayed tissue healing, cell apoptosis as well as tissue necrosis and subsequent chronic bacterial infection can occur due to the limited oxygen delivery. The microenvironment of the damaged wound is highly hypoxic due to the high oxygen consumption and disruption of vascular. Prolonged or chronic hypoxias may increase the risk of inflammation and infection, delays tissue healing and may ultimately lead to tissue necrosis. Thus, the proper oxygen level is critical for wound healing process. Adequate oxygen supplement can induce angiogenesis, encourages cell differentiation, increases cell proliferation and migration, enhances extracellular matrix (ECM) synthesis, and promotes wound contraction[1]. In order to address the challenges of oxygen deprivation and bacterial infection in wound healing, the purpose of the study is to develop a multifunctional injectable tissue engineering scaffold that could 1) provide a sustained oxygen release over an extended period of time to increase cell proliferation and 2) provide bacterial resistance against pathogens.

Methods:

Oxygen-generating injectable scaffold (OGIS) was prepared by encapsulating calcium peroxide (CPO)-based Poly-ε-caprolactone (PCL) microparticles into catalase-containing poly (N-isopropylacrylamide)-Chitosan (PNIPAM-CS) hydrogel.

Oxygen dissolved within the deionized water was measured under 1% oxygen to mimic hypoxic conditions. Rat skin fibroblasts were seeded in the OGIS, non oxygen-generating control scaffold and scaffold free control and cultured under hypoxic condition (1% O_2, 37 °C). Cell viability and cell proliferation were characterized by MTT assay.

Staphylococcus aureus (*S. aureus*, ATCC 6538, Gram-positive bacteria) and *Pseudomonas aeruginosa* (*P. aeruginosa*, ATCC 10145, Gram-negative bacteria) were used as the model strains for gram positive and negative pathogen to evaluate the antimicrobial function of the OGIS. The antimicrobial activity of OGIS was evaluated by modified Kirby-Bauer test and colony count antibacterial assay.

Results:

Scanning electron microscopy (SEM) images (Fig. 1) showed oxygen-generating microparticles was formed as well as the structure of PNIPAM-CS hydrogel. According to Figure 2, no significant burst release was observed and the OGIS demonstrated a sustained oxygen release.

MTT test for cell proliferation revealed that the OGIS showed a significantly higher cell number compared to the control.

Clear zones of inhibition around OGIS were observed on both *S. aureus* and *P. aeruginosa* Kirby-Bauer test. In contrast, control group does not have any inhibitory effect on the growth of *S. aureus* and *P. aeruginosa*. In addition, the result of colony count antibacterial assay demonstrated that the OGIS was provide a significant inhibition on the growth of *S. aureus* and *P. aeruginosa* after a certain contact time, while the control scaffold shows no antimicrobial activity under the same condition.

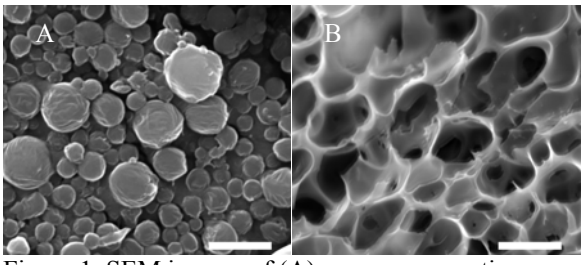

Figure 1. SEM images of (A) oxygen-generating microparticles; (B) The structure of PNIPAM-CS hydrogel. Scale bar represents 5 μm.

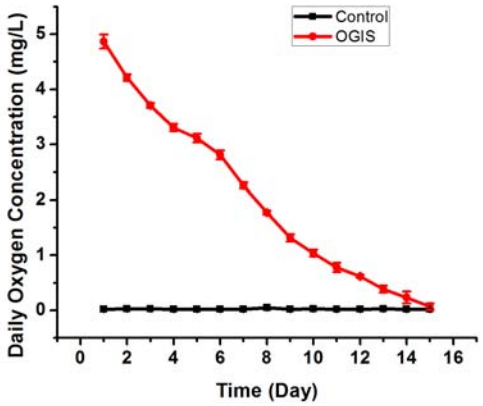

Figure 2. Oxygen release kinetic of the OGIS when incubated under hypoxic conditions (1% O_2) at 37 °C. An equal amount of microparticles without CPO was used as a control. Each data point represents n= 3, mean ± SD

Conclusions:

Oxygen-generating microparticles and PNIPAM-CS injectable hydrogel were successfully fabricated. The OGIS can be injected and gelled *in situ* at 37 °C to fill tissue defects and provide a long-term oxygen delivery, enhance cell proliferation under hypoxic wounded environments, while simultaneously against bacterial infection or improve the existing chronic infection conditions. All of the results suggested that the OGIS can be designed and developed as wound healing scaffold.

References:

[1] S. Guo, L. A. Dipietro, Journal of dental research 2010, 89, 219.