Effect of Oxygen Generating Materials on Biodegradable Scaffolds

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Introduction

Regeneration of large three-dimensional tissues faces a limitation of insufficient oxygen and nutrient transport inside a structure that is larger than the limit for oxygen diffusion, which is a few hundred microns. To maintain cell viability inside a scaffold it is important that the cells have a constant oxygen supply that can allow time for blood vessels to perfuse through the whole construct. Ideally, a supplemental source of oxygen would be present in the scaffold while vasculature becomes incorporated. Our group has previously shown that an oxygen generating scaffold could function as this source of oxygen [1]. The next approach to analyzing oxygen generating scaffolds is to investigate how the oxygen generating material, peroxide-based particles, integrates with a general polymer matrix.

It is hypothesized that the oxygen generating material can be incorporated into several biodegradable polymer scaffolds, exhibiting versatility for several applications. These scaffolds will be analyzed for their of oxygen releasing behavior, structure, mechanical properties and biocompatibility to determine the relevant applications for use.

Methods

❑ ***Formation of Oxygen Generating Scaffolds***

Scaffolds were fabricated with a solvent casting/particulate leaching procedure, using the combinations listed in Table 1. For the oxygen generating scaffold, calcium peroxide particles (CPO) was added at 5 wt% to the polymer solution and mixed well. Polymer solutions were mixed with paraffin particles (300 – 500 μm), transferred to petri plates and frozen. Scaffolds were punched out with a 8 mm biopsy punch and precipitated in cold EtOH. Paraffin was leached out in hexane and the scaffolds were vacuum-dried. SEM images were taken to reveal the porous scaffold structure and Trypan blue staining was performed to show the distribution of the CPO material in the scaffolds.

Table 1: Scaffold compositions

Polymer	Solvent	Oxygen Generating Particle
PDLGA	DMSO	CPO
PLLA	HFP	CPO
PGA	HFP	CPO
PCL	DMSO	CPO

❑ ***Mechanical Testing of Scaffolds***

Mechanical properties were assessed on scaffolds with and without CPO (Ø ~ 7 mm, T ~ 6 mm). The compressive strength was determined from the maximum load at 90% deformation and the compressive modulus was calculated from the linear part of the stress-strain curve.

❑ ***Oxygen Generation of Scaffolds***

The oxygen release behavior of the scaffolds was measured with a pH/Dissolved oxygen (DO) meter (Thermo Scientific). Each scaffold was wetted and then placed separately in 40 ml nitrogen purged PBS. Measurements were done at room temperature, in a hypoxic glovebox system calibrated to a 0.4 % oxygen level.

❑ ***In vitro cell culture***

To evaluate whether all scaffolds could support cell proliferation NIH3T3 mouse fibroblasts were seeded on all constructs. Scaffolds were incubated in an hypoxic environment (0.2% O_2, 5% CO_2) at 37 °C. At 1 and 3 days, an MTS assay was performed to assess the cell viability.

Results

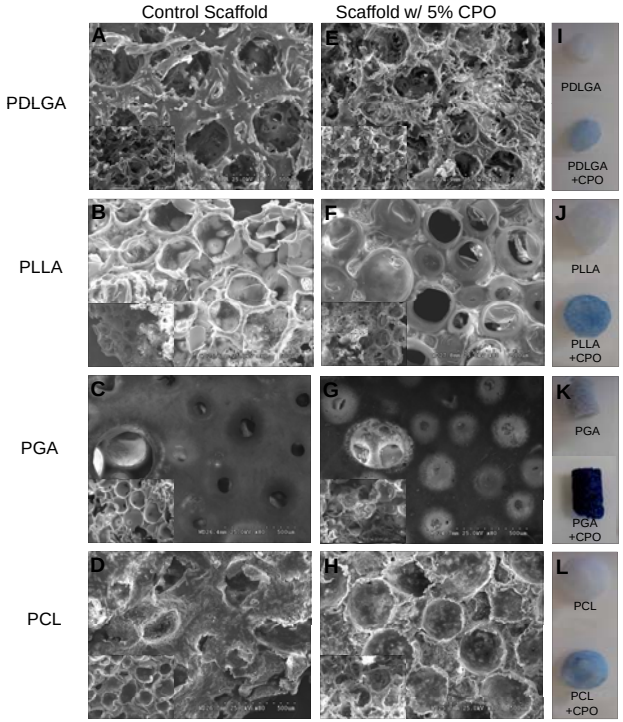

Figure 1: *SEM images A-D show surfaces of control scaffolds, inset shows cross-section of same scaffolds. SEM images E-H shows the surfaces for the CPO containing scaffolds. All images taken at 80x. Figures I-L show macroscopic images of the scaffolds stained with Trypan blue to visualize the distribution of the CPO throughout the scaffold.*

Polymer	Compressive strength (MPa)	Compressive modulus (MPa)
PDLGA	0.6 ± 0.5	0.16 ± 0.04
PDLGA/CPO	1.4 ± 0.6	0.2 ± 0.2
PLLA	0.8 ± 0.3	0.18 ± 0.04
PLLA/CPO	0.4 ± 0.2	0.09 ± 0.05
PGA	1.3 ± 0.3	0.5 ± 0.2
PGA/CPO	0.6 ± 0.3	0.4 ± 0.1
PCL	0.9 ± 0.1	0.28 ± 0.06
PCL/CPO	1.0 ± 0.3	0.6 ± 0.4

Table 2: *Compressive mechanical properties of control scaffolds and oxygen generating scaffolds with 5% CPO were performed with a compression interface of 50 mm on a Instron Model 5544. A 100N load cell was used at a cross-head speed of 0.4 mm/min and measurements taken at 90% deformation. Results are presented as means ± standard deviations (n=5). Data was analyzed for outliers with a q-test and data points were rejected with 95% confidence.*

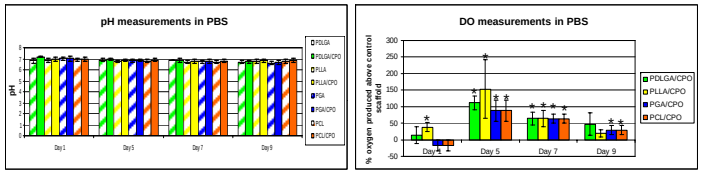

Figure 2: *pH and DO measurements in PBS in hypoxic environment at RT. The PBS buffers any potential increase in pH that could arise from the CPO material. The level of dissolved oxygen is higher for all the CPO scaffolds compared to control scaffolds at most time points. Error bars designate standard deviations and significant differences p<0.05 is indicated with * (n=3).*

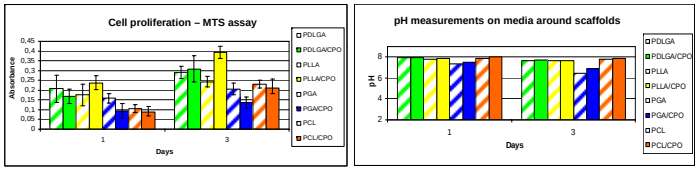

Figure 3: *The MTS assay shows that all scaffolds support cell growth and viability over a period of 3 days inside an hypoxic environment. The media around each scaffold type was collected and the pH measured to control that pH stayed within physiological values. Error bars designate standard deviations (n=3).*

Conclusions

Scaffolds composed of various polymers showed a porous structure with similar cross-sections of 300-500 μm pores. However, the surfaces of the constructs differed in morphology, which could affect cell infiltration as well as degradation. The compressive strength and modulus values of the scaffolds were comparable to the theoretical polymer properties, however more testing has to be performed before matching the scaffolds with specific applications. All polymers were able to produce oxygen generating scaffolds, where the CPO scaffolds produced significantly higher levels of oxygen compared to the control scaffolds. The scaffolds stayed within the physiological pH values and any increase in pH that could occur when the CPO comes into contact with water was buffered by the PBS. All scaffolds could support cell growth and viability in a hypoxic environment over 3 days, while maintaining a physiological pH.

It has been shown that an oxygen generating material could be successfully incorporated into scaffolds produced from PDLGA, PLLA, PGA and PCL using a standard, repeatable fabrication method. Differences in mechanical properties of the scaffolds combined with the ability of all scaffolds to produce oxygen and support cell growth would indicate a large area of use for this novel oxygen generating material.

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References

1. Oh S, *et* al. Biomaterials, 2009; 757-762