Perfluorinated molecules as novel oxygen vectors for tissue engineering

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Introduction

Fabricating engineered tissue will remain a challenge until oxygen delivery to cells seeded on scaffolds is improved. This is exemplified by results showing cellular spheroids containing a hypoxic and necrotic center surrounded by a rim of viable cells.[1] Previous attempts to overcome these limitations relied on perfusion bioreactors where oxygen dissolved in the culture medium diffuses to the scaffold interior.[1] However, the high flow rate required to maintain adequate oxygen concentration for cell viability often surpasses the shear stress tolerance of cells.[1] Alternatively, perfluorocarbon (PFC) emulsions have been investigated as oxygen carriers. PFCs have a high dissolving power for oxygen but are hydrophobic, lipophobic, and stable which makes them biologically compatible. Unlike oxygen chemically bound to hemoglobin, solubilized oxygen can be rapidly and extensively extracted from perfluorinated molecules when needed.[1] However, the high density of these emulsions results in settling of the droplets. It would therefore be advantageous if the oxygen carrier molecules were embedded in the scaffold.

Scaffolds with embedded perfluorinated oxygen vectors will enhance the amount of oxygen available to the cells seeded on them. As a result, cells will show enhanced infiltration in the scaffolds due to the increased availability of oxygen at greater depths thus improving the viability of engineered vascular tissues. The objective of this study is to design cardiovascular tissue engineering scaffolds bearing different perfluorinated oxygen vectors, specifically perfluorinated poly(vinyl alcohol) (PVA) and cyclodextrin molecules.

PVA has been shown to be biocompatible and can provide mechanical stability and flexibility. It has been used in numerous biomedical applications, including as the base material for electrospun scaffolds. This molecule presents a viable opportunity for modification with PFCs as it contains numerous hydroxyl groups. Cyclodextrins, cyclic oligosaccharides made up of 6-8 glucose units, have been shown to be bio-compatible, stable, and non-toxic to cells and are currently employed as safe pharmaceutical excipients. The unique shape of cyclodextrin molecules allows them to readily form inclusion compounds (ICs) with a number of hydrophobic species.

Materials & Methods

In this study, inclusion compounds were formed with perfluorodecalin, a hydrophobic compound, by vigorously stirring measured amounts of perfluorodecalin in a saturated aqueous solution of β-cyclodextrin for 72h. The resulting white, crystalline precipitate was filtered and washed with cold water and acetone and dried under vacuum at room temperature for 24h. Characterization of the product was done with attenuated total resonance Fourier transform infrared spectroscopy (ATR-FTIR).

PVA was perfluorinated with the acid chloride perfluorooctanoyl chloride using the synthesis previously developed by Zhu *et al*.[2] Briefly, PVA (87-89% hydrolyzed) was dissolved in dry dimethylformamide (DMF) at 150°C until transparent and then reacted with perfluorooctanoyl chloride at 100°C for 10h with pyridine as a catalyst. The product was precipitated in methanol, filtered, washed with hot toluene, and dried under vacuum at 60°C. The product could not be characterized by F^{19} NMR as it was not soluble in typical NMR solvents[3] so ATR-FTIR was used.

Results

A comparison of the IR spectra for the perfluorodecalin/β-cyclodextrin system and the unmodified β-cyclodextrin molecule reveal a number of differences in the 4000-3000cm^{-1} region. The small differences in the OH frequencies between the IC and β-cyclodextrin can be attributed to interactions with the guest molecule.[4] In addition, the different spectroscopic signals in the fingerprint region confirm that the perfluorodecalin/β-cyclodextrin system is different from the unmodified β-cyclodextrin.

IR analysis of the PVA product compared to unmodified PVA showed that the hydroxyl stretching vibration in the unmodified PVA had decreased significantly indicating successful synthesis.[3] In addition, carbonyl stretching was observed at 1730cm^{-1} for the perfluorinated sample.

Discussion

The design of novel oxygen delivery strategies is critical for developing viable solutions to repair damaged tissues. The development of novel oxygen vectors has been investigated with the aim of incorporating the products directly in scaffolds which will be characterized with respect to morphological properties by scanning electron microscopy. Finally, the effect of the embedded oxygen vectors will be studied with respect to cell toxicity and ability to support cell growth throughout the scaffold using human coronary artery smooth muscle cells.

References

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