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Microbubbles as biocompatible porogens for hydrogel scaffolds

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ABSTRACT

In this study, we explored the application of lipid-shelled, gas-filled microbubbles as a method for creating on-demand microporous hydrogels for cartilage tissue engineering. The technique allowed for homogenous distribution of cells and micropores within the scaffold, increasing the absorption coefficient of large solutes (70 kDa dextran) over controls in a concentration-dependent manner. The stability of the gas phase of the microbubbles depended on several factors, including the initial size distribution of the microbubble suspension, as well as the temperature and pressure during culture. Application of pressure cycles provided controlled release of the gas phase to generate fluid-filled micropores with remnant lipid. The resulting microporous agarose scaffolds were biocompatible, leading to a twofold increase in engineered cartilage properties (E_Y = 492 ± 42 kPa for the bubble group vs. 249 ± 49 kPa for the bubble-free control group) over a 42-day culture period. Our results suggest that microbubbles offer a simple and robust method of modulating mass transfer in cell-seeded hydrogels through mild pressurization, and the methodology may be expanded in the future to include focused ultrasound for improved spatio-temporal control.

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1. Introduction

Designing biocompatible scaffolds with architecture that optimizes solute transport is challenging for fields such as cartilage tissue engineering. If scaffold architecture is too dense, nutrient supply becomes insufficient and cells die or underperform [1]. However, if scaffold porosity is too high, cell products are lost to the bathing culture medium to the detriment of tissue development. An ideal tissue scaffold would provide a balance between a local pore structure sufficient to retain cell products that form extracellular matrix (ECM) tissue and a global architecture that provides adequate nutrient supply to cells at the central core regions of the scaffold. This is a difficult challenge because the cross-sectional thickness of cartilage varies considerably across the human joint (ranging from 1 to 6 mm across the patella), so engineers must design scaffolds that optimize solute transport across a wide range of diffusion distances. Secondly, the scaffold architecture itself is transformed with culture time by cell-mediated synthesis of ECM, so the diffusivity of some important solutes (such as growth factors) is reduced over time. In short, the spatio-temporal development of these nutrient gradients must be accounted for when designing scaffolds for larger construct applications.

Agarose has been used extensively in cartilage biology [2–5] and is currently being evaluated in human clinical trials as a scaffold component of a next-generation autologous chondrocyte implantation strategy [6,7]. We have found that 2% agarose (Type VII, Sigma) permits robust cartilaginous tissue growth with Young's moduli (E_Y) and glycosaminoglycan (GAG) levels similar to that of native tissue after ⩽8 weeks in culture [8]. This robust growth, however, is limited to small cylindrical constructs. We have observed that constructs of >1 mm thickness develop a "U-shaped" axial distribution of properties beginning at ~2 weeks, with central regions having less matrix elaboration relative to stiffer peripheral regions [9]. The development of inhomogeneous tissue properties with culture time is presumably due to nutrient limitations at the core region. From Fick's law, the diffusion time of a solute is proportional to the square of the distance (distance^2 $\propto$ diffusivity $\times$ time), so even small changes in scaffold thickness can have major impacts on tissue growth.

As with most hydrogels, the pore size of agarose decreases with polymer concentration [1]. Researchers are therefore examining methods to increase nutrient availability while maintaining the polymer concentration and local pore size (~∅232 nm) [1] that was found to be effective in the development of small engineered constructs (volume: 0.0126 ml). Recently, we obtained successful

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