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ABSTRACT TEMPLATE

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"*Chlorella*-enriched hydrogels exhibit a protective role against myocardial damage by reducing reactive oxygen species in an *in vitro* model of ischemia/reperfusion using cardiac spheroids"

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Microalgae are promising photosynthetic microorganisms for biofabricating advanced biomaterials, improving oxygenation and decreasing reactive oxygen species (ROS) production. However, their use in engineering human tissues has been limited due to their intrinsic growth requirements, which are not compatible with human cells. In this study, we generated alginate-gelatin (AlgGel) hydrogels with varying densities of *Chlorella vulgaris*, and we evaluated whether microalgae may have caused any change in hydrogels' mechanical and structural properties. Moreover, we evaluated their effects on cardiac cells' pathophysiological response under both normoxic and ischemia/reperfusion (I/R) conditions. For this, we used cardiac spheroids (CSs), which include the most representative human cardiac cell types (cardiac endothelial cells, myocytes, and fibroblasts) and approximate the complex human heart microenvironment. Our findings showed that the addition of *Chlorella* did not alter the mechanical properties of AlgGel containing between of 10^5 cells mL^{-1} and 10^6 cells mL^{-1} ,based on our pore size measurements, as well as by Brillouin microspectroscopy and rheological testing. Consistent with the reduction in pore size measured in hydrogels containing 10^7 *Chlorella* cells mL^{-1}, we measured a reduction of CS viability after 14 days in culture under normoxic conditions, while CS viability was not altered in hydrogels containing either 10^5 *Chlorella* cells mL^{-1} or 10^6 *Chlorella* cells mL^{-1}. Following the induction of hypoxia/reoxygenation of CSs using our I/R model, we demonstrated that the *Chlorella*-enriched hydrogels protected cardiac cells against I/R-induced reduction in intracellular oxygen levels and viability, which was dependent on reactive oxygen species generation. Additionally, the addition of algae to I/R CSs protected against their loss in contractile function, with a significant increase in the contraction frequency in Chlo5 and Chlo6 hydrogels. Altogether, our findings demonstrated that *Chlorella*-enriched hydrogels offer a novel therapeutic option for mitigating ischemia/reperfusion (I/R) injury through enhanced ROS scavenging activity and support their use as promising biomaterials for cardiac tissue engineering for a quick translation from bench to bedside.

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