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O_2-controllable hydrogels for studying cellular responses to hypoxic gradients in three dimensions *in vitro* and *in vivo*

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Abstract:

Oxygen (O_2) acts as a potent upstream regulator of cell function. In both physiological and pathophysiological microenvironments, the O_2 concentration is not uniformly distributed but instead follows a gradient that depends on distance from oxygen-carrying blood vessels. Such gradients have a particularly important role in the development, tissue regeneration, and tumor growth. In this protocol, we describe how to use our previously reported gelatin-based O_2-controllable hydrogels that can provide hypoxic microenvironments *in vitro*. The hydrogel polymeric network is formed via a laccase-mediated cross-linking reaction. In this reaction, laccase catalyzes diferulic acid (diFA) formation to form hydrogels with an O_2-consuming reaction. Cells, such as cancer or endothelial cells, as well as tumor/tissue grafts, can be encapsulated in the hydrogels during hydrogel formation and then analyzed for cellular responses to 3D hypoxic gradients and to elucidate the underlying mechanisms governing these responses. Importantly, oxygen gradients can be precisely controlled in standard cell/tissue culture conditions and *in vivo*. This platform has been applied to study vascular morphogenesis in response to hypoxia and to understand how oxygen gradients mediate cancer cell behavior. Herein, we describe the means to validate the assay from polymer synthesis and characterization – which take 1 – 2 weeks and include verification of ferulic acid (FA) conjugation, rheological measurements, and O_2 monitoring – to the study of cellular responses and use in rodent models. Time courses for biological experiments using this hydrogel are variable, and thus they may range from hours to weeks, depending on the application and user end goal.

Keywords: oxygen gradients, O_2-controllable hydrogel, hypoxic microenvironment, 3D hypoxic gradients, vascular morphogenesis, cancer cell behavior