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Hydrogel formulation determines cell fate of fetal and adult neural progenitor cells

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Abstract Hydrogels provide a unique tool for neural tissue engineering. These materials can be customized for certain functions, i.e. to provide cell/drug delivery or act as a physical scaffold. Unfortunately, hydrogel complexities can negatively impact their biocompatibility, resulting in unintended consequences. These adverse effects may be combated with a better understanding of hydrogel chemical, physical, and mechanical properties, and how these properties affect encapsulated neural cells. We defined the polymerization and degradation rates and compressive moduli of 25 hydrogels formulated from different concentrations of hyaluronic acid (HA) and poly(ethylene glycol) (PEG). Changes in compressive modulus were driven primarily by the HA concentration. The in vitro biocompatibility of fetal-derived (fNPC) and adult-derived (aNPC) neural progenitor cells was dependent on hydrogel formulation. Acute survival of fNPC benefited from hydrogel encapsulation. NPC differentiation was divergent: fNPC differentiated into mostly glial cells, compared with neuronal differentiation of aNPC. Differentiation was influenced in part by the hydrogel mechanical properties. This study indicates that there can be a wide range of HA and PEG hydrogels compatible with NPC. Additionally, this is the first study comparing hydrogel encapsulation of NPC derived from different aged sources, with data suggesting that fNPC and aNPC respond dissimilarly within the same hydrogel formulation.

Introduction

The high water content and highly customizable nature of hydrogels make these materials well suited for tissue engineering, especially in the brain and spinal cord. Unfortunately, too many studies use hydrogels without reporting the tunable properties. As a consequence, they may not recognize how these properties can alter their final results. Hydrogel chemistry, including the molecular weight (mw) of the

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components, the type of polymerizing modifications, and the total polymer content, all contribute to the customization of hydrogel behaviors (e.g. the physical properties, such as polymerization and degradation, and the mechanical properties). These behaviors will determine the success of a hydrogel used for tissue engineering. For example, two studies treating spinal cord injury used chemically similar hydrogels but reported substantially different results: Park et al. (2010) found significant repair after spinal cord injury using a hyaluronic acid (HA; mw 170 kDa) hydrogel with an identified shear storage modulus (G′) of 0.3 kPa, but an undefined final weight percent (wt.%) of HA and an unspecific HA modification to allow for polymerization. In contrast, Horn et al. (2007) found no repair of the spinal cord using a thiol-modified HA-based hydrogel at a 0.5 or 1.0 wt.%, but failed to report the molecular weight of the HA or the mechanical properties of the hydrogel. While the hydrogels used in these two studies are very similar in chemistry, neither study provides enough detail about the tunable properties to be independently replicated. Hydrogel chemistry and subsequent physical and mechanical properties all have unique contributions to successful tissue engineering, specifically with regard to the reaction of the host tissue to the hydrogel and how replacement cells respond to hydrogel encapsulation (Aurand et al., 2012a,b). A comprehensive exploration of hydrogels well suited for neural tissue engineering, composed of commercially available materials with defined tunable properties may help standardize the use of hydrogels for neural tissue repair.

Hydrogels comprised of HA and poly(ethylene glycol) (PEG) provide both the natural element (HA) for neural cell interaction and the synthetic element (PEG) for customization and functionalization. HA is the main polymer backbone of the extracellular matrix (ECM) of the brain and is degraded by hyaluronidases produced by both neurons and glia in vivo (Al'Qteishat et al., 2006; Lindwall et al., 2013). Biologically inert PEG provides additional control over hydrogel physical properties and helps to enhance functionality through prefabrication of more complex polymers, allowing for the attachment of cells or incorporation of growth factors or drugs (Aurand et al., 2012a,b; Burdick et al., 2006; Lampe et al., 2011; Lin and Anseth, 2009; Lin et al., 2009, 2011; Sawhney et al., 1993; Young and Engler, 2011). Our study employs only these two components, without further modifications, to assess baseline biocompatibility and explore how changes in hydrogel polymer ratio and subsequent physical and mechanical properties affect the fate of encapsulated neural progenitor cells (NPC).

Many studies have explored the use of neural cells and tissue for their use in treating neurological disorders (Goldman, 2011; Barker et al., 2003; Svendsen et al., 1999). Clinical trials have been undertaken implanting neural stem cells and NPC to treat a number of neurological diseases, including Parkinson's, Batten disease, and cerebral palsy (Gupta et al., 2012; Olanow et al., 2003; Trounson et al., 2011; Selden et al., 2013; Luan et al., 2012). Because of the ethical controversies surrounding the destruction of a fetus, research has also begun to explore the potential use of NPC derived from adult brain (Lo and Parham, 2009; Su et al., 2011). Currently published studies often treat NPC derived from fetal or adult brains as the same type of cells. Indeed, Pollard et al. (2006) found NPC from adult and fetal sources express the same neural progenitor markers (e.g., nestin, sox2, blbp and olig2) and respond similarly to basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF). While the major molecular NPC qualities are common to both cell types, few studies have compared the fates of these two cell types in vitro and even fewer studies have addressed the fate of hydrogel-encapsulated NPC using cells from either source.

Hydrogel biocompatibility is improved when the mechanical properties of the hydrogel are matched to the host tissue (Aurand et al., 2012a,b; Banerjee et al., 2009; Engler et al., 2006; Georges et al., 2006; Saha et al., 2008; Seidlits et al., 2010; Teixeira et al., 2009). Since tissue-matching is an important quality of a successful hydrogel, we investigated the fate of both fetal- and adult-derived NPC in twenty-five different HA and PEG hydrogels. Because fetal-derived NPC are extracted from a brain with an inherently weaker mechanical integrity than adult-derived NPC, where the brain is stiffer (Elkin et al., 2010), we hypothesized that the survival and differentiation of NPC would be optimal when encapsulated into hydrogels that more closely match the originating tissue. Our study is unique because of this comparison between fetal tissue-derived NPC and adult tissue-derived NPC encapsulated in a range of biologically relevant hydrogels with mechanical properties of both juvenile and adult brain.

Methods

Hydrogel formulations and polymerization

Polymer hydrogels were formulated from thiol-modified carboxy-methylated hyaluronic acid (CMHAS; "HA") and thiol-reactive poly(ethylene glycol) diacrylate (PEG). Both products were purchased from Glycosan BioSystems, Inc. (Cat.# GS222 and GS700). The HA had a molecular weight of 250 ± 30 kDa and a company-reported degree of methylation of approximately 75–85%; the PEG had a molecular weight of 3.4 kDa and the company has reported a 95% or greater degree of acrylation. Lyophilized HA and PEG solids were reconstituted with degassed, deionized water (DG water; cat# GS241, Glycosan BioSystems, Inc.) to 2%(w/v HA)

Table 1 Matrix of 25 hydrogels studied. Final hydrogel formulations based on the HA-to-PEG ratio were designated by the letters A–Y. For example, the hydrogel identified as formulation "M" had a final polymer concentration of 0.6%(w/v) HA and 1.8%(w/v) PEG. HA: hyaluronic acid, PEG: poly(ethylene glycol), wt%: percent weight by volume of polymer in final hydrogel formulation.

		HA (wt%)				
		0.2	0.4	0.6	0.8	1.0
PEG (wt%)	3.0	U	P	K	F	A
	2.4	V	Q	L	G	B
	1.8	W	R	M	H	C
	1.2	X	S	N	I	D
	0.6	Y	T	O	J	E