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A Hyaluronan-Based Injectable Hydrogel Improves the Survival and Integration of Stem Cell Progeny following Transplantation

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SUMMARY

The utility of stem cells and their progeny in adult transplantation models has been limited by poor survival and integration. We designed an injectable and bioresorbable hydrogel blend of hyaluronan and methylcellulose (HAMC) and tested it with two cell types in two animal models, thereby gaining an understanding of its general applicability for enhanced cell distribution, survival, integration, and functional repair relative to conventional cell delivery in saline. HAMC improves cell survival and integration of retinal stem cell (RSC)-derived rods in the retina. The pro-survival mechanism of HAMC is ascribed to the interaction of the CD44 receptor with HA. Transient disruption of the retinal outer limiting membrane, combined with HAMC delivery, results in significantly improved rod survival and visual function. HAMC also improves the distribution, viability, and functional repair of neural stem and progenitor cells (NSCs). The HAMC delivery system improves cell transplantation efficacy in two CNS models, suggesting broad applicability.

INTRODUCTION

Cell transplantation in the central nervous system (CNS) requires exogenous cells to survive and integrate into the neural circuitry, thereby restoring function. The three major barriers to successful cell transplantation in adult tissue are distribution, survival, and integration of donor cells. The co-dependency of cell survival and cell integration on transplantation efficacy has been described (Ma et al., 2011).

Targets for cell therapy in the CNS, including retina and brain, have tissue-specific challenges that must be overcome for successful treatment. In conditions such as age-related macular degeneration and retinitis pigmentosa, transplanted outer retinal cells may be able to use the remaining inner retinal circuitry, and thus photoreceptor replacement is a feasible strategy to promote functional repair of the retina (Klassen et al., 2004). Although functional restoration after subretinal cell transplantation of neonatal or embryonic stem cell (ES)-derived postmitotic rods into adult hosts has been demonstrated (Pearson et al., 2012; Lamba et al., 2009), the majority of studies have reported relatively low survival, from 0.04% to 8% on average. Similarly, in the brain, transplanted stem cells typically show low survival of 2%–8% (Nakagomi et al., 2009). Biomaterial approaches show promise in improving the efficiency of cell transplantation.

The hyaluronan (HA) and methylcellulose (MC) (HAMC) hydrogel is injectable, minimally swelling, bioresorbable, and fast gelling (Gupta et al., 2006; Baumann et al., 2010). It was shown to be superior to a number of different natural polymers in terms of physical and biological properties, including support of stem cell progeny survival and proliferation (Mothe et al., 2013; Ballios et al., 2010). The fast-gelling properties of HAMC are key to the more uniform distribution of cells in the retina and brain compared to conventional saline delivery techniques.

The intimate relationship between cell survival and integration is investigated here with transplants of retinal stem cell (RSC)-derived rod photoreceptors. The development and characterization of adult RSC-derived rods in vitro (Ballios et al., 2012) closely resemble newborn post-mitotic rod photoreceptors in vivo (Akimoto et al., 2006), with expression of first immature (Nrl+ [Neural retina leucine zipper+]) and then mature (Rhodopsin+) rod markers in RSC progeny treated with taurine and retinoic acid (taurine/RA). Twelve-day in vitro differentiated rods ("immature" RSC-derived rods) express high levels of Nrl and low levels of Rhodopsin, whereas 28-day in vitro differentiated rods ("mature" RSC-derived rods) express high levels of both Nrl and Rhodopsin. Importantly, RSC-derived rods display electrophysiologic and functional light responsiveness in vitro that is similar to immature rod photoreceptors (Demontis et al., 2012). Transplantation of RSC-derived

photoreceptors has demonstrated functional repair in early post-natal mouse models of disease (Inoue et al., 2010).

The role of HAMC in cell survival, integration, and, ultimately, functional repair was investigated in the retina with RSC-derived rods and in the brain with neural stem and progenitor cells (NSCs). In both tissues, cells delivered in HAMC survived significantly better than those delivered in conventional buffered saline vehicles. This survival effect was mediated by cell-material interactions through CD44, the putative HA receptor, and confirmed in vivo when transplanted CD44−/− RSC-derived rods no longer responded to the pro-survival effect previously observed with HAMC. In the retina, disruption of the outer limiting membrane (OLM) with DL-α-aminoadipic acid (AAA) (West et al., 2008) enhanced migration/integration of cells into the host outer nuclear layer (ONL). When delivered in HAMC, these integrated cells adopted mature rod morphology, expressed mature rod markers, and improved visual function in genetically blind mice. Unexpectedly, optimization of the delivery vehicle and host environment is sufficient to promote integration of mature rods, a population of cells previously considered unsuitable for transplantation (Pearson et al., 2012; MacLaren et al., 2006).

To gain greater insight into the broad applicability for cell delivery, HAMC was investigated for the delivery of adult mouse NSCs (Morshead et al., 1994) to the brain. Significantly more cells were observed when delivered in HAMC versus artificial cerebrospinal fluid (aCSF) controls. Moreover, the depth of penetration and cell distribution were superior when NSCs were delivered in HAMC, underlining the benefit of HAMC for cell-host tissue interaction. Most important, the enhanced cell survival observed for cells delivered in HAMC versus aCSF correlated with significant behavioral recovery in the endothelin-1 (Et-1) model of stroke: only animals that had cells delivered in HAMC showed functional repair.

This study underscores the importance of the biomaterial to successful cell transplantation, where HA promotes cell survival and MC promotes cell distribution. An injectable hydrogel delivery strategy that promotes cell survival and integration of transplanted adult stem cell progeny shows promise as a strategy for cell replacement in the retina and brain for functional repair.

RESULTS

HAMC Improves Overall Survival of RSC-Derived Post-Mitotic Rods following Transplantation

RSC-derived rods at various stages of maturation were transplanted into the subretinal space of adult albino CD10 mice (Figure 1) and evaluated for survival 3 weeks post-transplantation. The survival of undifferentiated (0-day) RSCs delivered in saline and fully differentiated (44-day) RSC-derived rods showed the poorest survival (Figure 1A), whereas the differentiated progeny (between 12 and 28 days) showed improved survival. The greatest survival was observed for the mature (28-day) RSC-derived rods transplanted in HAMC. This survival rate was significantly greater than delivery in saline, suggesting a stage-specific interaction between mature RSC-derived rods and HAMC (two-way ANOVA, vehicle and differentiation time interaction, $F(5,40) = 3.37$, $p < 0.05$). Compartmental analysis of cell distribution in host retinal tissue (Figures 1B–1D for neural retina, subretinal space, and retinal pigment epithelium [RPE] layer, respectively) using three-way ANOVA revealed a three-way interaction among differentiation time, delivery vehicle, and compartment on cell survival ($F(10,120) = 6.19$, $p < 0.05$). Committed immature RSC-derived rods (12-day differentiated) showed a greater percentage of integrated cells into neural retina compared to mature rods, regardless of delivery vehicle (post hoc analysis, $p < 0.05$) (Figure 1B). Significantly fewer cells remained in the subretinal space among immature rods (12 days) transplanted in HAMC versus saline ($p < 0.05$) (Figure 1C), demonstrating their ability to migrate into retinal tissue. Interestingly, independent of the number of days of taurine/RA differentiation, a large fraction of transplanted cells were adherent to, rather than integrated in, the RPE layer of the retina (Figure 1D), where cells were counted in the RPE compartment if they were in contact and adherent to this layer. Transplants of immature rods extended short processes following integration (Figures 1E and 1F), whereas those with mature rods in HAMC integrated into the ONL and extended processes toward the outer plexiform layer and subretinal space, compared to saline (Figures 1G and 1H). When completely undifferentiated (0-day) cells were transplanted, most cells were located in the RPE layer, and almost no cells were in the subretinal space or neural retina (Figures 1B–1D). These data are consistent with previous studies (Ballios et al., 2010).

To determine whether fusion with host cells occurred with transplanted RSC progeny, undifferentiated GFP-positive (*Actin.gfp*) RSC progeny were transplanted into adult transgenic mice that ubiquitously express mRFP (*Actin.mrfp1*). Confocal image analysis showed that GFP and mRFP signals did not overlap (n = 4 independent transplants; Figure S1). In addition, transplants of undifferentiated *Pax6α-Cre* RSC progeny into *Z/EG* pups (P2) showed strong expression of the lacZ reporter in the host retina. In the case of cell fusion, GFP expression due to Cre-mediated recombination would have been expected; however, no GFP-positive cells were observed (n = 4 independent transplants; data not shown). On the basis of this evidence, it is unlikely that transplanted RSC progeny fused with host cells.