PHENOTYPIC AND FUNCTIONAL ASSESSMENT OF MAGNETICALLY LABELED PIG EMBRYONIC STEM CELL DERIVED HEPATOCYTES

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INTRODUCTION: For many severe, progressive liver diseases the only effective treatment is liver transplantation. Unfortunately, due to the shortage of available donor organs, liver transplantation is not available to all patients. Metabolic liver diseases, particularly loss-of-function phenotypes affecting children, may be amenable to creative alternative treatments, such as hepatocyte transplantation. Xenotransplantation of swine cells to humans as a short-term measure is an emerging concept in regenerative medicine. Effective non-invasive imaging of transplanted cells is essential to advance this effort. Here we describe our investigation into the phenotypic and functional properties of magnetically labeled pig embryonic stem cell-derived hepatocytes for MRI-based cell tracking of regenerative liver therapy.

PICM-19 cells are bipotent liver parenchymal cells derived from pig embryonic stem cells (reviewed in Talbot et al., J. Anim. Sci., 2013). PICM-19 hepatocytes exhibit serum protein production, inducible cytochrome P450 activity, γ-glutamyltranspeptidase (GGT) activity, ammonia clearance and urea production. PICM-19 cholangiocytes self-organize into3D, multicellular ductules resembling bile ductules cultured from fetal or adult pig liver. The PICM-19 ductules express GGT at their apical cell surfaces and exhibit transcellular fluid transport to ductal lumens with *in vivo*-like kinetics in response to physiological levels of secretin. PICM-19 cells are promising for swine regenerative medicine models of liver disease and for potential human xenotransplantation.

MATERIALS AND METHODS:

PICM-19 hepatocytes were grown within a collagen/Matrigel sandwich in mouse STO fibroblast-conditioned medium. After 2 d in culture, cells were labeled 24 h by incubation with 100 particles per cell of 0.96 micron-sized iron oxide particles (MPIOs). Assays performed included 2D gel and MS analysis of serum-free conditioned medium (CM) for secreted protein identification, GGT histochemistry, forskolin and glucagon responsiveness, mitotic index, light and electron microscopy.

RESULTS and DISCUSSION: PICM-19 cells labeled with MPIOs (> 80%), comparable to previous work (Bennewitz, et al., Mol Im Biol, 2012). The morphology and ultrastructure were unaffected by the presence of the MPIOs. 2D gel analysis of CM showed normal serum-proteins, but also regucalcin, a cytoplasmic protein. GGT histochemistry showed intense staining at the biliary canaliculi between the cells. Forskolin and glucagon induced the expansion of the canaliculi via cAMP-dependent transcellular fluid transport. Mitotic index of MPIO-labeled and control cells were similar (0.6%). TEM analysis showed that labeled cells remain unmaligned with respect to cellular appearance and morphology.

These experiments confirm that magnetic labeling of PICM-19 hepatocytes with MPIOs does not impact the phenotypic or functional properties of these cells, paving the way for their use in MRI-based cell tracking of liver regenerative medicine paradigms. Further, these experiments demonstrate the usefulness of functional assays, rather than detection of cell surface CD markers for studying effects of magnetic particles on labeled cells.

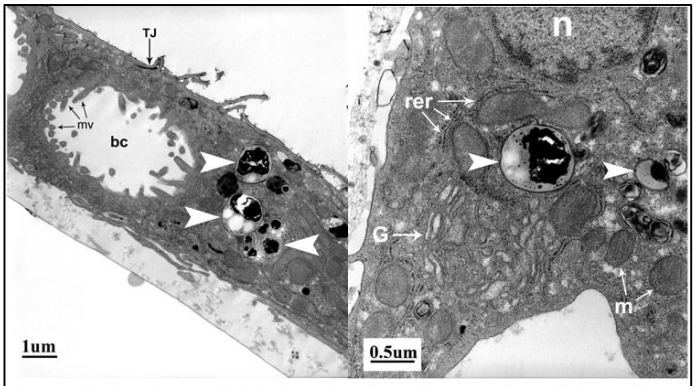
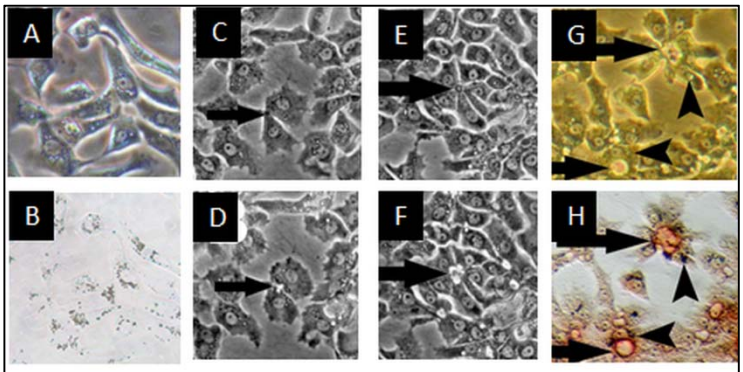


Figure 2: TEM of labeled PICM hepatocytes. **Left**: mv = microvilli (projecting into the lumenal space of the biliary canaliculi), TJ = tight junction (seals the biliary canaliculi so that fluid cannot escape into the extracellular environment), bc = biliary canaliculi (specialized junction between hepatocytes which in vivo channel fluids to the bile ducts – as they leave each liver lobules, white arrowheads indicate the membrane bound iron particles taken up by the cells. **Right**: n = nucleus, rer = rough endoplasmic reticulum, G = Golgi complex, m = mitochondria, white arrowheads indicate the iron particles, both of which are membrane bound. The morphology of the cell and its organelles appear unaffected by the presence of the magnetic particles.



Phenotypic and functional assays of magnetically labeled PICM-19 hepatocytes. A) Phase contrast and B) dark field microscope image of labeled cells. C) Before and D) after images of forskolin response, showing the expansion of a canaliculus from the cAMP-induced transcellular fluid transport. E) Before and F) 20 minutes after glucagon was added. An expanded biliary canaliculus is shown with black arrows. G) and H) GGT stained after the addition of glucagon. G) is phase-contrast, 200x, and H) is the same area photographed with Hoffman modulation 200x. Note the intense GGT histochemical staining at the apical cell membrane surfaces surrounding the biliary canaliculi (arrows). Arrowheads indicate iron particles.

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