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Primary human hepatocytes on biodegradable poly(l-lactic acid) matrices: A promising model for improving transplantation efficiency with tissue engineering

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Abstract: Liver transplantation is an established treatment for acute and chronic liver disease. However, because of the shortage of donor organs, it does not fulfill the needs of all patients. Hepatocyte transplantation is promising as an alternative method for the treatment of end-stage liver disease and as bridging therapy until liver transplantation. Our group has been working on the optimization of matrix-based hepatocyte transplantation. In order to increase cell survival after transplantation, freshly isolated human hepatocytes were seeded onto biodegradable poly(l-lactic acid) (PLLA) polymer scaffolds and were cultured in a flow bioreactor. PLLA discs were seeded with human hepatocytes and exposed to a recirculated medium flow for 6 days. Human hepatocytes formed spheroidal aggregates with a liver-like morphology and active metabolic function. Phase contrast microscopy showed increasing numbers of spheroids of increasing diameter during the culture period. Hematoxylin and eosin histology showed viable and intact hepatocytes inside the spheroids. Immunohistochemistry confirmed sustained hepatocyte function and a preserved hepatocyte-specific cytoskeleton. Albumin, alpha-1-antitrypsin, and urea assays showed continued production during the culture period. Northern blot analysis demonstrated increasing albumin signals. Scanning electron micrographs showed hepatocyte spheroids with relatively smooth undulating surfaces and numerous microvilli. Transmission electron micrographs revealed intact hepatocytes and junctional complexes with coated pits and vesicles inside the spheroids. Therefore, we conclude that primary human hepatocytes, precultured in a flow bioreactor on a PLLA scaffold, reor ganize to form morphologically intact liver neotissue, and this might offer an optimized method for hepatocyte transplantation because of the expected reduction of the initial cell loss, the high regenerative potential in vivo, and the preformed functional integrity.

Statement: Our study reports on the first success of growing human liver tissue from primary human liver cells on resorbable scaffolds made from material similar to surgical sutures. This liver tissue could be used in place of donor organs during liver transplantation or during the bridge period until a suitable donor is available for patients with acute liver failure. Thousands of patients are on the waiting list to receive a suitable liver. Liver cell transplantation offers a possible solution in overcoming the organ shortage. In addition, liver cells have excellent regenerative potential making liver cell transplantation a viable therapeutic approach for patients with metabolic defects or fulminant hepatic failure as the native liver is preserved while liver dysfunction may resolve as regeneration occurs. Dr. Eva Török and her collaborative team of four UKE institutions and one US collaborator isolated liver cells from 12 human liver specimens with a viability of 82%. After a two-day culture period, liver cells formed tightly packed cellular aggregates, called spheroids, and took on a liver-like appearance. Human liver cells were distributed across a three-dimensional porous structure of the polymer scaffolding. From day two to four, the average number of spheroids more than doubled from 18 to 41 per visual field. "Our experimental model represents a promising technique to culture human liver cells and prepare them for transplantation on a biodegradable polymer scaffold into the peritoneal cavity. Further studies are underway to confirm our results and may ultimately offer viable clinical options for liver cell transplantation in the future." A related editorial also published in the same issue of Liver Transplantation acknowledges the huge clinical potential for liver cell transplantation.

The research team from four UKE institutions (Hepatobiliary and Transplant Surgery, Internal Medicine I, Medical Microbiology, Virology and Hygiene, and Anatomy II - Experimental Morphology) has a long collaborative tradition in the field of hepatocyte related research with the focus on tissue engineering, hepatocyte transplantation and liver regeneration as well as viral hepatitis studies. The work was partially funded by a study grant of the European Society for Organ Transplantation (to Dr. Eva Török) and the Werner-Otto-Stiftung (to PD Dr. Dr. Jörg-Matthias Pollok).