eneration Using a Three Dimensional Hepatic atrix

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ptions for the treatment of end stage liver disease. The is liver transplantation but organ donor shortage is a h little hope of improvement in the foreseeable future. specific biologic scaffolds composed of extracellular omise for the constructive remodeling of organs such as bladder, and skin. The present study involves the whole rat livers by retrograde perfusion. The remaining matrix, when continuously perfused, retains its three nd structure, including microvasculature. Seeding of the caffold with primary hepatocytes has shown maintenance ferentiation and the retention of hepatocellular function by albumin production, EROD metabolism and ammonia epatic ECM scaffolds were recellularized using three an effort to determine which method was optimal. Sheets ocytes with morphologically normal appearing cell to cell observed following recellularization and in-vitro culture. stitute functional three-dimensional hepatic parenchyma regenerative medicine therapy for patients with end stage

416 Expression of Liver Specific Functions in Rat Hepatocyte Spheroid Array

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It is known that spherical multicellular aggregates (spheroids), which are formed by the rearrangement and compaction of a cell aggregates, have a tissue-like structure and can express higher levels of liver-specific functions as compared to the traditional hepatocyte monolayer. In this study, we developed a spheroid array system and evaluated the expression of genes encoding key molecules involving in liver-specific functions, namely, cell adhesion molecules, transcription factors, protein and metabolic enzymes, and transporters.

The hepatocyte spheroid array chip was manufactured by combination of the microfabrication and the microcontact printing. The chip comprised 672 circular microwells in a triangular arrangement; each well was 300 μm in diameter, 200 μm in depth, and 400 μm in pitch. The center of each microwell had a 100- μm cell adhesion area was modified with collagen, and the remaining part of each microwell was modified with polyethylene glycol to create the non-adhesive area. The hepatocytes cultured on the chip gradually aggregated on the collagen-coated area in each microwell and had developed into a smooth spheroid had a uniform diameter. Though the expression levels of all the genes encoding key molecules in the spheroids tended to decrease gradually with culture time, they were consistently higher than those in the monolayer culture for at least 10 days of culture.

These results suggest that hepatocyte spheroids acquire intercellular organization and maintain many metabolic functions. Thus, this hepatocyte spheroid array system seems to be a promising model for various *in vitro* cell-based assays.

hree-Dimensional Primary Hepatocyte Cul- in-based Hydrogel

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tes, a main cell type in liver, carry out most of the ons of liver, including production of majority of the eins, regulation of the carbohydrate, urea, and lipid ll as detoxification of exogenous chemicals. However, atocytes rapidly lose their functions and viability after s is focused on the maintenance of their function for a erm in *in vitro* primary hepatocyte culture. Formation cytes in 3-D is one of the approaches for a long-term se of hydrogels is a promising way because of their t, and the easy tuning of their modulus to that of the ously, we developed an in situ forming heparin-based e gelled in the presence of biomolecules, such as cells med by a Michael-type addition reaction between and diacrylated poly (ethylene glycol). Heparin is ing with numerous ECM proteins and growth factors -binding domain. Particularly, heparin is one of the ides in liver, and it has a high affinity for hepatocyte GF), well known as a potent factor in growth and ocytes. In this study, we applied the heparin-based epatocyte culture. The initial viability of hepatocytes on was sufficiently high (over 70%), and the eir viability as well as functions over several weeks The effects of adding HGF, using spheroids of applying co-culture with stellate cell also will be

418 Mesenchymal Stem Cell Therapy Ameliorates Acute Liver Injury in Rabbits

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Acute liver failure is characterized by rapid deterioration of liver function and a high mortality. Currently, there are no specific therapies except liver transplantation. However, shortage of donor organs remains the major obstacles. Cell therapy of acute liver failure should provide rapid support for the injured liver. However, adult human hepatocytes have a poor proliferative potential. Bone marrow comprising heterogeneous cell populations contains certain progenitors with the ability to differentiate into multiple mesenchymal cell lineages. To identify any differentiation plasticity of adult bone marrow mesenchymal stem cells (MSCs), we used allyl alcohol (AA) treated rabbits for an acute liver failure model.

The MSCs were aspirated and expanded to a cellular concentration of 1×10^8/4 ml. The rabbits were injected AA intraperitoneally. Three days after the injection, the MSCs were injected via portal vein. The control group rabbits were injected with 4 ml of normal saline under the same condition. The postoperative recovery was closely monitored by hepatic function markers. Decreased portal fibrosis after stem cell injection was noted by Masson's trichrome stain, while the reticulin framework restoration was also vaguely identifiable by reticulin stain.

Whether extracorporeal devices or the transplantation of primary hepatocytes, stem cells or cells genetically engineered to over-express key metabolic functions, a proliferative phenotype or cytoprotective pathways will be best suited to meeting these demanding challenges.