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## Implanted subcutaneous bioscaffold seeded with hepatocyte-like cells, an approach for hemophilia treatment

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**Background**: Hemophilia A is a congenital X-linked bleeding disorder resulting from deficiency of coagulation factor VIII. Hemophilia A affects one in 5000 male births. This disorder is characterized by recurrent bleeding episodes, primarily into the joints and muscles. Over time, repeated joint bleeding damages the cartilage and bone. Hemophilia is typically treated with coagulation-factor replacement. However, there are many complications of factor replacement therapy as neutralizing anti-FVIII antibody (inhibitor) development as well as the burden of care. Recent studies confirmed the ability of a number of human cell types to secrete FVIII. Our previous published work established the ability of bone marrow derived stem cells to be induced to secrete FVIII. Recently, we can implant subcutaneously in an experimental rats the generated bioscaffold from piglet liver seeded it with human stem cells transdifferentiated into hepatic-like cells to secrete human FVIII.

Aim of Work: The aim of the study is to evaluate the ability of the generated bioscaffold which implanted subcutaneously in experimental rats to secrete human FVIII.

**Materials & Methods**: Experimental design include 6 adult male albino rats which were divided into control where rats were left untreated and implanted groups where the generated bioscaffold were implanted subcutaneously in the abdominal wall and all rats were sacrificed after 10 days.

## Generation of Bioscaffold:

- 1. Surgical procedure: A 5 kilogram piglet liver was removed by intraoperative ligation of the hepatic artery and splenic vein to avoid leakage of the detergents used in decellularization process.
- 2. Decellularization of pig liver: 15 liters of distilled water was injected in to the liver until complete blood inside the liver was washed and then 6 liters of detergent was injected (1% SDS + 0.1% ammonium hydroxide) to kill the hepatocytes and the endothelial cells. Decellularized parts were detected by being transparent then special lab techniques were performed to confirm that these parts are completely decellularized.
- 3. Cryopreservation of decellularized liver was followed by cutting using cryomicrotome and then punch biopsy.
- 4. Seeding of discoid scaffolds with transdifferentiated hepatocyte-like cells.
- 5. Implantation of generated organoid subcutaneously.

Evaluation was done by laboratory investigation which includes detecting human FVIII in the serum of rats. And histological examination was done by examining sections from the scaffold by H&E stain and CD19 immunohistochemistry for stem cells.

**Results & Conclusions**: This study proves the ability of the generated bioscaffold which implanted subcutaneously in experimental rats to secrete human FVIII in the serum of experimental rats. H&E examination showed hepatocytes like cells in the implanted scaffold. CD19 were positive for stem cell.

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