Acta Biomaterialia 10 (2014) 3209–3216

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Reduced liver cell death using an alginate scaffold bandage: A novel approach for liver reconstruction after extended partial hepatectomy

Eyal Shteyer [a,b,*], Ami Ben Ya'acov [a], Lidia Zolotaryova [a], Avital Sinai [a], Yoav Lichtenstein [a], Orit Pappo [c], Olga Kryukov [d], Tsiona Elkayam [d], Smadar Cohen [d,e,*], Yaron Ilan [a]

[a] Liver Unit, Hebrew University – Hadassah Medical Center, Jerusalem, Israel
[b] Pediatric Gastroenterology Unit, Department of Pediatrics, Hebrew University – Hadassah Medical Center, Jerusalem, Israel
[c] Department of Pathology, Hebrew University – Hadassah Medical Center, Jerusalem, Israel
[d] Avram and Stella Goldstein-Goren Department of Biotechnology Engineering, Ben-Gurion University, Beer Sheva, Israel
[e] Regenerative Medicine and Stem Cell (RMSC) Research Center, Ben-Gurion University, Beer Sheva, Israel

ARTICLE INFO

Article history:
Received 3 July 2013
Received in revised form 17 February 2014
Accepted 25 February 2014
Available online 5 March 2014

Keywords:
Alginate scaffolds
Liver
Regeneration
Extended partial hepatectomy

ABSTRACT

Extended partial hepatectomy may be needed in cases of large hepatic mass, and can lead to fulminant hepatic failure. Macroporous alginate scaffold is a biocompatible matrix which promotes the growth, differentiation and long-term hepatocellular function of primary hepatocytes in vitro. Our aim was to explore the ability of implanted macroporous alginate scaffolds to protect liver remnants from acute hepatic failure after extended partial hepatectomy. An 87% partial hepatectomy (PH) was performed on C57BL/6 mice to compare non-treated mice to mice in which alginate or collagen scaffolds were implanted after PH. Mice were scarified 3, 6, 24 and 48 h and 6 days following scaffold implantation and the extent of liver injury and repair was examined. Alginate scaffolds significantly increased animal survival to 60% vs. 10% in non-treated and collagen-treated mice (log rank = 0.001). Mice with implanted alginate scaffolds manifested normal and prolonged aspartate aminotransferases and alanine aminotransferases serum levels as compared with the 2- to 20-fold increase in control groups ($P < 0.0001$) accompanied with improved liver histology. Sustained normal serum albumin levels were observed in alginate-scaffold-treated mice 48 h after hepatectomy. Incorporation of BrdU-positive cells was 30% higher in the alginate-scaffold-treated group, compared with non-treated mice. Serum IL-6 levels were significantly decreased 3 h post PH. Biotin-alginate scaffolds were quickly well integrated within the liver tissue. Collectively, implanted alginate scaffolds support liver remnants after extended partial hepatectomy, thus eliminating liver injury and leading to enhanced animal survival after extended partial hepatectomy.

1. Introduction

Of all the organs, the liver is unique in its ability to repair itself after suffering loss of tissue mass from toxins, infectious agents or surgical resection. Partial hepatectomy in humans is often needed and well tolerated in the presence of primary or secondary liver tumors [1]. Nevertheless, there are cases in which extended partial hepatectomy is warranted due to large hepatic mass, but poses a high risk for fulminant hepatic failure [2]. Currently, liver transplantation is the only option in many cases of acute liver failure and end-stage liver disease. However, although great advances have been made in the past several decades, liver transplantation still leads to significant morbidity and mortality. Also, the complexity of liver structure and function, as well as the limited supply of donated livers, poses unique challenges.

Liver-tissue engineering aims to provide novel therapies and endeavors to replace liver transplantation for various liver diseases. Many natural and synthetic implantable tissue-engineering approaches utilize porous scaffolds, which are temporary structures that mimic the extracellular matrix (ECM). The scaffolds provide mechanical support and allow the adherence, proliferation and migration of hepatocytes [3,4]. In the last decade or so, hepatocytes have been encapsulated within various natural, ECM-derived scaffolds, including collagen gels [5,6], hyaluronic acid [7] and peptides [8] as well as in scaffolds fabricated from the anionic polysaccharide from algae, alginate.

* Corresponding authors at: Pediatric Gastroenterology Unit, Department of Pediatrics, Hebrew University – Hadassah Medical Center, Jerusalem, Israel. Tel.: +972 508946462; fax: +972 2 6778966 (E. Shteyer), Department of Biotechnology Engineering, Marcus Campus, Bldg 39 Rm222, Ben-Gurion University of the Negev, Beer Sheva, 84105 Israel. Tel.: +972 8 6461798; fax: +972 8 6472915 (S. Cohen).
E-mail addresses: eyals@hadassah.org.il (E. Shteyer), scohen@bgu.ac.il (S. Cohen).

http://dx.doi.org/10.1016/j.actbio.2014.02.047
1742-7061/

Macroporous alginate scaffolds with pore sizes of ~100 μm have been shown to support spheroid formation from primary rat and human hepatocytes because of the weak adhesive properties of alginate, and spheroid formation, in turn, promoted hepatocyte function stabilization [9–11]. Dvir-Ginzberg et al. have demonstrated in vitro that three-dimensional macroporous alginate scaffold with a pore size of 50–100 μm is an effective promoter of the maturation of newborn hepatocytes into a functional hepatic tissue capable of maintaining prolonged hepatocellular function [12]. Another study showed in rats that vascularization of porous alginate scaffolds implanted on liver lobes led to improved hepatocyte engraftment [13].

These studies led us to investigate the macroporous alginate scaffolds, solely, with no implanted cells, as biocompatible matrices to support the liver regeneration process. We found in an 87% partial hepatectomy (PH) model that alginate scaffolds were sufficient for support of the liver remnants by providing a replacement milieu for the remaining liver cells, thus reducing cell necrosis, improving hepatic synthetic functions and improving regeneration, which together led to enhanced animal survival after extensive partial hepatectomy.

2. Materials and methods

2.1. Macroporous scaffold preparation

Macroporous alginate and collagen scaffolds with similar porous structures as judged by scanning electron microscope images (90% porosity, 50–100 μm pore size, 0.04 cm^3 volume) were utilized [12]. The scaffolds were prepared in a 96-well plate. The alginate scaffolds were prepared from 1% (w/v) sodium alginate (LVG (100 kDa), 65% G, NovaMatrix, FMC Biopolymer, Drammen, Norway) solution cross-linked with 0.22% (w/v) D-gluconic acid (hemi-calcium salt) by a freeze-dry technique [14]. Calf skin collagen (Sigma–Aldrich, Cat # C9791) scaffolds were prepared from a 1% (w/v) collagen solution in 0.2% (v/v) acetic acid and were then freeze-dried under the same conditions used for preparing alginate scaffolds. The collagen scaffolds were further subjected to ultraviolet cross-linking to enhance stability.

2.2. Animals and surgical procedures

Male C57BL/6 mice, weighing 25–28 g, were used in all experiments and were maintained in a temperature-controlled room with alternating 12 h light/dark cycles. A standard two-thirds hepatectomy comprises removal of the lateral left, medial left, and medial right lobes. To achieve 87% PH we further resected the left lateral, the pyriform process of the liver sparing only the caudate lobe [15]. After hepatectomy, three disc-shaped scaffolds (10 mm diameter, 3 mm thick) per animal were positioned on top of the liver remnant (caudate lobe). The capacity of the macroporous alginate scaffolds to enhance liver reconstruction after PH was compared to macroporous collagen scaffolds and also to a non-treated group. The disc-shaped collagen scaffolds were similar in porous structure and in the same size as the alginate scaffolds; and were different only by their chemical nature [12].

To evaluate the survival after PH, mice (n = 3–4 per group) were implanted with alginate or collagen scaffolds and were compared to hepatectomized mice without any treatment (non-treated group). To analyze liver remnant tissue and serum, mice in all groups were scarified at 3, 6, 24, 48 h and 6 days after hepatectomy. The experiments were repeated three times. All experiments were approved by the Institutional Animal Care and Use Committee of Hadassah-Hebrew University Hospital. Animals received human care according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals.

2.3. Liver histology and immunohistochemistry

Mice were killed at various time points after PH, and liver remnants were then collected and fixed in 10% formalin. Liver morphology and histology were assessed in 5 μm hematoxylin and eosin (H&E)-stained paraffin sections. Sections were visualized by light microscopy (×100). Alginate scaffolds could be detected in some H&E-stained liver sections under the light microscope in the polarized light mode, due to their higher refractive index.

To detect the implanted scaffolds within the liver, traceable scaffolds with similar macroporous structure were prepared with biotin-labeled alginate. The biotin was covalently coupled to alginate, as described with details by Polyak et al. [16]. The alginate biotin-labeled scaffolds were implanted in hepatectomized mice, at various time points as described above for regular alginate or collagen scaffolds. For the detection of biotin-labeled scaffolds within the liver after PH, 5 μm sections of paraffin-embedded liver specimens was used. Sections were stained using the DAKO-ARK kit (Dako, Denmark) except that biotinylated primary antibody was not used for the first step of staining. Briefly, after endogenous peroxidase blocking, sections were washed and incubated with streptavidin peroxidase for 15 min and then followed with DAB incubation, all according to the manufacturer's instructions. Tissue ingrowth into biotin-labeled scaffold was analyzed under a bright field microscope (Nikon ECLIPSE E600, Tokyo, Japan) with several magnifications (×10, ×40 and ×200) and images were captured by a computer-assisted morphometric image system (Cell A software). Hepatocyte nuclear staining for BrdU was performed essentially as described [17]. The frequency of nuclear BrdU labeling in hepatectomized mice (48 h after PH) was determined by the examination of 10 random ×100 fields and at least 300 cells and nuclei in each tissue section were counted. Animals received an intraperitoneal injection of 30 μg BrdU per g body weight at 2 h prior to euthanasia. After euthanasia, liver specimens were obtained and processed for histological staining and BrdU analysis. Four animals were used per treatment group for each time point and 10 separate low-power fields were evaluated per animal. Sectioned liver tissues were stained by using the cell proliferation kit, according to the manufacturer's instructions. The groups of treatments were blinded to the observer.

2.4. Liver enzymes and albumin

The effects of alginate and collagen scaffolds on liver injury were determined by measuring the enzymatic activity levels of AST and alanine aminotransferase (ALT) activities in the sera of all mice. These activities were measured using a Reflovet Plus clinical chemistry analyzer (Roche Diagnostics, GmbH, Mannheim, Germany). Serum albumin levels were determined by an automatic analyzer (Cobas Mira Plus, Roche Diagnostic, Branchburg, Germany).

2.5. Serum IL-6 levels

Serum levels of IL-6 were determined in various time points by a sandwich ELISA using a commercial kit according to the manufacturer's recommended instructions (Quantikine, R&D Systems, Minneapolis, MN, USA).

2.6. Statistical analysis

At least eight mice were analyzed per each time point or group described in the different experiments. Differences among groups