Video Article

Bioluminescence Imaging for Assessment of Immune Responses Following Implantation of Engineered Heart Tissue (EHT)

Lenard Conradi[1,2], Christiane Pahrmann[1], Stephanie Schmidt[1], Tobias Deuse[1,3], Arne Hansen[2], Alexandra Eder[2], Hermann Reichenspurner[1], Robert C. Robbins[3], Thomas Eschenhagen[2], Sonja Schrepfer[1,3]

[1]Transplant and Stem Cell Immunobiology Lab (TSI) and CVRC, University Hospital Hamburg, University Heart Center Hamburg

[2]Department of Experimental and Clinical Pharmacology and Toxicology, University Heart Center Hamburg

[3]CT Surgery, Stanford University School of Medicine

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Abstract

Various techniques of cardiac tissue engineering have been pursued in the past decades including scaffolding strategies using either native or bioartificial scaffold materials, entrapment of cardiac myocytes in hydrogels such as fibrin or collagen and stacking of myocyte monolayers [1]. These concepts aim at restoration of compromised cardiac function (e.g. after myocardial infarction) or as experimental models (e.g. predictive toxicology and substance screening or disease modelling). Precise monitoring of cell survival after implantation of engineered heart tissue (EHT) has now become possible using in-vivo bioluminescence imaging (BLI) techniques [2]. Here we describe the generation of fibrin-based EHT from a transgenic rat strain with ubiquitous expression of firefly luciferase (ROSA/luciferase-LEW Tg; [3]). Implantation is performed into the greater omentum of different rat strains to assess immune responses of the recipient organism following EHT implantation. Comparison of results generated by BLI and the Enzyme Linked Immuno Spot Technique (ELISPOT) confirm the usability of BLI for the assessment of immune responses.

Video Link

The video component of this article can be found at http://www.jove.com/video/2605/

Protocol

1. EHT Generation and Culture Conditions

Manufacture of fibrin-based EHT was performed as described elsewhere [4]. In brief, to generate fibrin-based EHT for implantation purposes, a master mix was prepared containing cells isolated from neonatal ROSA/luciferase-LEW transgenic rat hearts, bovine fibrinogen and Matrigel. Agarose casting molds were prepared using Teflon spacers placed in 6-well culture dishes. After solidification of agarose, spacers were removed and silicone post racks were placed on culture dishes with posts reaching into the molds. To generate EHT, the mastermix was briefly mixed with a calculated amount of thrombin and pipetted into the mold. After polymerisation of fibrinogen, silicone racks with EHTs adhering to the posts were gently removed from the agarose casting molds, transferred to new 6-well cell culture dishes and maintained in a 37°C, 7% CO_2 cell culture incubator using custom-made cell culture medium.

EHT were kept under cell culture conditions until day ten. During this time span, neonatal rat heart cells began to elongated, interconnect and align along force lines in between silicone posts. At the time of implantation, spontaneous coherent contractions were observed, deflecting silicone posts.

2. EHT Implantation

EHT implantation was performed in either immunocompetent Brown Norway (BN) rats, or immunodeficient nude rats to investigate the allogeneic immune response. BN and nude rats weighing 100 - 150 g were purchased from Charles River Germany (Sandhofer Weg 7, D-97633 Sulzfeld) and housed under conventional conditions, fed standard rat chow and water ad libidum. Immunodeficient rats are housed in sterile bedding, cages, and sterilized water is used. The German ethical review committee reviewed and approved the animal procedures. All surgical instruments are sterilized prior to use.

The implantation procedure are performed as follows:

Rat are anesthesized with isoflurane (2.5-3%) using an induction chamber. Human exposure to isoflurane can be reduced by working in a downdraft table or non-recirculating hood. Body temperature is maintained during the surgical procedure.

1. Shave abdominal area and apply eye ointment to prevent the eyes from drying during anesthesia.
2. Place the rat on its back and place a facemask over its nose and mouth to keep up the anesthesia.
3. Disinfect the abdominal area using Betadine and then 80% ethanol. Swab from clean to dirty, from center of shaved area moving outward toward the haired area.
4. Check reflexes pinching the hind feet to be sure that the rat is sufficiently anesthetized.
5. Drape the animal and perform a midline abdominal incision separating the skin and muscle in two steps to open the abdomen. Hot bead sterilization is used between skin and muscle opening.
6. Place the intestines in a warmed saline moisturized powder free glove. Fold the glove around the intestines to prevent loss of moisture.
7. Remove fatty tissue and expose the spleen.
8. Visualize and spread greater omentum with forceps.
9. Carefully cut EHT from suspending silicone posts and transfer onto the greater omentum.
10. Wrap the greater omentus around the EHT and fix using two single 7-0 prolene sutures (Ethicon, Norderstedt, Germany)
11. Next move the intestines back into the abdomen.
12. Flush the abdomen with prewarmed sterile saline.
13. Close the muscle layer of the abdominal wall using 6-0 prolene running sutures (Ethicon, Norderstedt, Germany).
14. Use 5-0 prolene (Ethicon, Norderstedt, Germany) running sutures to close the skin.
15. While the rat is still in anesthesia, inject 5 mg/kg Carprofen subcutaneously.
16. To provide sufficient analgesia for this type of procedure, an analgesic (such as Metamizol) is added to the drinking water (50 mg Metamizol per 100 ml) for pain medication for 3 days post transplantation.

3. Bioluminescence Imaging of EHT Following Implantation

The IVIS bioimaging platform (Xenogen, Alameda, CA, USA) is used for non-invasive bioluminescence imaging *in-vivo* and results are analyzed using the IVIS Living Image (Xenogen) software package. Imaging is performed daily starting 2 hours postoperatively.

Anesthetize rat with isoflurane (2.5 - 3%) using an induction chamber.

1. Disinfect the abdominal and thoracic area widely using Provo-Iodine, next use 80% ethanol, repeat this step three times.
2. Inject d-Luciferin (Xenogen; 375 mg/kg body weight) i.p.
3. Place anaesthetized rats into the IVIS imaging chamber (up to 4 rats can be analysed at the same time).
4. Assess signal intensity of luc+ cells inside EHT in units of photons/sec/cm^2/steridianin the region of interest (ROI) at baseline (120 minutes after implantation) and at days 1, 2, 3, 5, 7, 10, and at weeks 2, 3 and 4. Note the peak signal (appears around 20 minutes after d-Luciferin injection).

Create a graph (Excel) showing the signal intensity over time.

4. Correlation of BLI Results and ELISPOT

The cellular *in-vivo* immune responses in immunocompetent BN and immunodeficient nude rats were studied after EHT transplantation. The spleen of recipient animals was harvested 5 days after EHT transplantation to isolate recipient splenocytes. Elispot assays using mitomycin-inhibited EHTs (Sigma Aldrich, St. Louis, MO, USA) as stimulator and 5 x10^6 recipient splenocytes as responder cells were performed according to the manufacturer's protocol (BD Biosciences) using IFN-γ and IL-4 coated plates to investigate TH1-, and TH2 response, respectively. IFN-γ- and IL-4 spots were significantly higher in the immunocompetent, model compared to the immunodeficient model (IFN-γ: p= 0.001; IL-4: p=0.023; Student's t-test).

5. Representative Results:

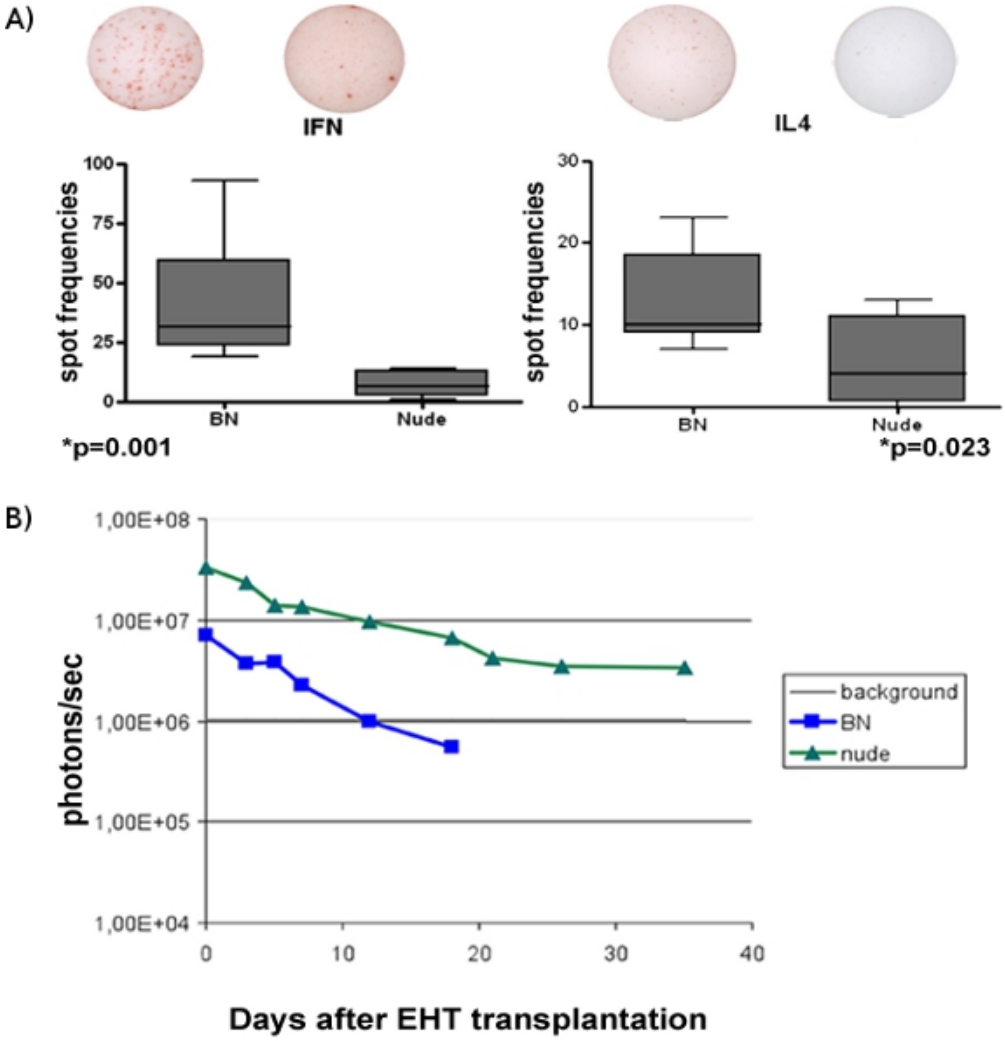

Figure 1. Correlation of BLI results and ELISPOT a) The cellular in vivo immune responses in immunocompetent Brown Norway and immunodeficient nude rats were studied after EHT implantation. Recipient splenocytes were harvested 5 days after EHT implantation. IFN? and IL-4 expression was evaluated by ELISpot assays using mitomycin-inhibited EHT as stimulators and 5x106 recipient splenocytes as responder cells. Th1 and Th2 responses were significantly higher in the immunocompetent model compared to the immunodeficient model as evidenced by IFNγ- and IL-4 production respectively. b) Luc+ EHTs were transplanted into the greater omentus of either immunocompetent BN rats or immunodeficient nude rats. Cell survival was longitudinally followed by BLI. The rate of animals that rejected the transplanted EHTs is presented. All BN rats rapidly rejected the EHT transplants, whereas the cells survived in T cell deficient rats.

Discussion

Before clinical implementation of EHT technology for cardiac repair, fundamental questions such as immunogenicity of both cellular and matrix components or graft survival after implantation need to be assessed in experimental models. *In-vivo* bioluminescence imaging represents a useful new technique for monitoring cell survival after transplantation. We successfully generated fibrin-based EHT containing luciferase positive (luc+) heart cells by adaptation of an established protocol [4]. These EHT were implanted into the greater omentum of recipient rats. Intraperitoneal injection of the specific substrate (d-Luciferin) generates signals that were detectable using the IVIS bioimaging platform (Xenogen). Signal intensity at baseline (120 minutes after implantation) measured in photons/sec/cm^2/steridian served as a surrogate parameter for the number of cells inside grafted EHTs. Daily measurements allowed for non-invasive longitudinal assessement of graft survival. In a rejection model, decline of signal intensity over time was correlated with the intensity and kinetics of acute rejection in different rat strains.

Disclosures

No conflicts of interest declared.

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