Tissue and Cell 49 (2017) 383–392

Contents lists available at ScienceDirect

Tissue and Cell

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

Polypropylene mesh seeded with fibroblasts: A new approach for the repair of abdominal wall defects in rats

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ARTICLE INFO

Article history:
Received 17 August 2015
Received in revised form 7 April 2017
Available online 17 April 2017

Keywords:
Polypropylene mesh
Abdominal wall defects
Fibroblasts
Wistar rat

ABSTRACT

Purpose: The purpose of study was to develop bioengineered scaffolds by seeding primary mouse embryo fibroblast cells (p-MEF) on polypropylene mesh and to test its efficacy for the repair of abdominal wall defects in rats.
Methods: The study was conducted on 18 clinically healthy adult Wistar rats of either sex. The animals were randomly divided into two equal groups having nine animals in each group. In both the groups a 20 mm × 20 mm size full thickness muscle defect was created under xylazine and ketamine anesthesia in the mid-ventral abdominal wall. In group I the defect was repaired with polypropylene mesh alone and in group II it was repaired with p-MEF seeded polypropylene mesh. Matrices were implanted by synthetic absorbable suture material (polyglycolic acid) in continuous suture pattern. The efficacy of the bio-engineered matrices in the reconstruction of full thickness abdominal wall defects was evaluated on the basis of macro and histopathological observations.
Results: Macroscopic observations revealed that adhesions with skin and abdominal viscera were minimum in group II as compared to group I. Histopathological observations confirmed better fibroplasia and collagen fiber arrangement in group II. No recurrence of hernia was found in both the groups.
Conclusion: Hernias are effectively repaired by implanting polypropylene mesh. However, this work demonstrates that *in vitro* seeding of mesh with fibroblasts resulted in earlier subsidization of pain, angiogenesis and deposition of collagen, increased thickness of matrices with lesser adhesions with underlying viscera. On the basis of the results p-MEF seeded mesh was better than non-seeded mesh for repair of abdominal wall defects in rats.

1. Introduction

The repair of large, complex abdominal wall defects remains significant clinical challenge. The integrity of abdominal wall may be lost due to several causes like trauma, infection, poor nutrition, and resection or over exertion which can lead to herniation of various abdominal organs. The cause for abdominal wall defect may be congenital or acquired. After abdominal surgery, incisional hernia is common and its repair is associated with high risk of complications like adhesions and recurrence (Franklin et al., 2003; Demir et al., 2005; Malangoni and Rosen, 2007; Penttinen and Grönroos, 2008). Two main predisposing factors of incisional hernia are infection and mechanical factors (Pailler et al., 1999). Complications of wound healing may lead to hernia especially when absorbable sutures have been used (Stock, 1954). In humans gastroschisis and omphalocele are found to be the common abdominal wall defects having significant morbidity and mortality (Wilson and Johnson, 2004).

Size, location and reducibility are the factors which decide the method of treatment of abdominal wall defect. In moderate sized easily reducible hernias conventional muscle to muscle apposition proves to be successful. But for the repair of large abdominal wall defects where abdominal pressure is high, supporting materials is required to reduce tension at the suture region (Park and Lakes, 1992). The surgical procedure for reconstruction of abdominal wall defects has gone through a series of changes. Previously abdominal wall defects were reconstructed by simply apposing muscles, later came the era of synthetic prosthetics and meshes. The use

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http://dx.doi.org/10.1016/j.tice.2017.04.004
0040-8166/

of polymer meshes in hernia surgery is one therapy of choice, in particular in recurring or large hernias. One of the most commonly used biomaterials in hernia repair is polypropylene (Kjeldsen and Gregersen, 1986; Nagy et al., 1996). The most frequent complication leading to implant failure and recurrence is infection of the surgical site (Ingle-Fehr et al., 1997). For avoiding these complications, the prosthetic material should be inert, and should also support fibroplasia (Johnson, 1969). Polypropylene is a strong, inert material that usually suffices, however, propensity for inducing extensive visceral adhesions and erosion of the skin or intestines are major drawbacks. These complications have encouraged the continuous search for new approaches and better materials (Bleichrodt et al., 1993).

Fibroblast cells play an important role in regeneration of new tissue and accelerating growth of tissue cells by secreting several growth factors and ECM. A major issue in tissue engineering especially in cases of *in vitro* cell culture-based studies is the identification of a suitable cell source and the ability to control proliferation and differentiation. Primary mouse embryonic fibroblasts (p-MEFs) have a number of properties making them an attractive cell culture model. Compared to other primary explant cultures they are easy to establish and maintain, proliferate rapidly resulting in large number of cells produced from a single embryo (Garfield, 2010). Major histocompatibility complex (MHC) class II antigens are present on the transplanting cells which are responsible for graft rejection. Fibroblasts lack these surface molecules and this makes them relatively immunologically inert. Seeding of fibroblasts on the biomaterials not only improves healing time but also the cosmetic appearance (Lamme et al., 2000). Fibroblasts constitute a potential autogenous source of cells for abdominal wall tissue engineering because they play a key role in-growth factor secretion, matrix deposition, and matrix degradation. Fibroblasts participate in wound healing by their ability to secrete prodigious quantities of ECM proteins and responding to and synthesizing cytokines, chemokines, and other mediators of inflammation (Eckes et al., 2000; Gabbiani, 2003). In addition, populations of fibroblasts have been shown to differentiate to become myofibroblasts that can exert contractile force due to the expression of myosin and alpha-smooth muscle actin, enabling wound area reduction. Fibroblast isolates have also been shown to contain progenitors able to differentiate into neurons and muscle cells *in vitro* (Toma et al., 2001) that show high levels of plasticity. Dermal fibroblast populations have been described as having progenitor cells with properties similar to that of mesenchymal stem cells (Boonen and Post, 2008). Furthermore, dermal fibroblasts are an easily accessible cell source without significant donor site morbidity and are easily expanded through *in vitro* culture.

In light of these possible advantages the present study was designed to develop a new approach by seeding primary mouse embryo fibroblast cells (p-MEF) on polypropylene mesh and to test the efficacy of this developed bioengineered mesh in the repair of abdominal wall defects in rats.

2. Materials and methods

2.1. Primary mouse embryo fibroblasts culture

Primary mouse embryo fibroblasts culture (p-MEF) was done as per standard protocol. The cells obtained were washed twice with Dulbeco's modified Eagle's medium (DMEM) containing antibiotics and were centrifuged at 2500 rpm for 8–10 min. The cells were re-suspended in cell growth media (DMEM-Low glucose) containing 10% FBS and antibiotics (mixture of 100 units/ml penicillin and 100 mcg/ml streptomycin). The cells were counted using a Neubaur's counting chamber method and plated at an average of 2.2×10^5 cells/cm^2 in T-25 flasks. They were maintained at 37 °C in a humidified atmosphere of 5% CO_2 in a CO_2 incubator. Day after the primary culture, the spindle shaped fibroblast cells were observed and the non-adherent/dead cells were removed by changing the medium. The cells were supplemented with fresh media to get a confluent monolayer of fibroblast cells. When the cells attained 80–90% confluency as assessed by visual inspection under inverted microscope, the cells were passaged into new culture flask. Culture medium was removed and cells were washed twice with HBSS (containing antibiotic). The cells were detached from the culture flask using 0.5% of trypsin-versene solution. The trypsin activity was stopped by adding equal volume of culture medium containing FBS and flushed properly to get the attached cells in suspension.

2.2. Seeding of p-MEF on polypropylene mesh

The desired sizes of polypropylene mesh were washed 4–5 times with antibiotics containing DMEM and were placed in wells of a 6 well tissue culture plate. The p-MEF cells were trypsinized to detach the monolayer of cells from the flask. Growth medium *i.e.* DMEM containing 10% fetal bovine serum was added and mixed properly to get single cell suspension. The cells were seeded on the mesh at the rate of 2×10^4 cells/cm^2. It was maintained at 37 °C in a humidified atmosphere of 5% CO_2 in a CO_2 incubator. The growth media was changed after 48 h. The seeded biomatrices were observed and processed for morphological assessment on third day post-seeding.

2.3. Testing the efficacy of bioengineered scaffolds for the repair of full thickness abdominal wall defects in rat model

The efficacy of bioengineered matrix *i.e.* p-MEF seeded polypropylene mesh was tested in the reconstruction of full thickness abdominal wall defects. The bioengineered matrix was compared with the polypropylene mesh alone.

2.4. Experimental design

The study was conducted on 18 clinically healthy adult Wistar rats of either sex, procured from Laboratory Animal Resources, Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India. The animals were acclimatized for 7 days in the new environment prior to beginning of study, and were monitored daily. Standard diet and water *ad libitum* were provided throughout the study. Animals with weights between 250 and 300 g were selected. The animals were randomly divided into two equal groups having nine animals each. In both the groups a 20 mm × 20 mm size full thickness abdominal muscle defect was created under xylazine and ketamine anesthesia in the mid-ventral abdominal wall. In group I the defect was repaired with polypropylene mesh alone and in group II it was repaired with p-MEF seeded polypropylene mesh. Matrices were implanted by synthetic absorbable suture material (polyglycolic acid) in continuous suture pattern.

The efficacy of the bio-engineered matrices in the reconstruction of full thickness abdominal wall defects were evaluated on the basis of following parameters.

2.5. Clinical observations

It included general behavioral changes, feeding pattern and rectal temperature. Degree of swelling at on the surgical site was graded as 0–3 scale: 0 = no swelling, 1 = mild swelling, 2 = moderate swelling, 3 = severe swelling. The degree of exudation at the site of repair was graded as 1–4 scale: 1 = none (apparently dry wound), 2 = mild exudates (wound is moist, no oozing on pressing the wound), 3 = moderate exudates (wound is moist, slight oozing on pressing the wound), 4 = extreme exudates (exudates is visible and