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Abdominal wall regenerative medicine for a large defect using tissue engineering: An experimental study

Masaya SUZUHIGASHI, Tatsuru KAJI, Kazuhiko NAKAME, Motoi MUKAI, Waka YAMADA, Shun ONISHI, Koji YAMADA, Takafumi KAWANO, Hideo TAKAMATSU, Satoshi IEIRI

Department of Pediatric Surgery, Research Field in Medical and Health Sciences,
Medical and Dental Area, Research and Education Assembly,
Kagoshima University, Kagoshima, JAPAN

Name of Sponsor: Satoshi IEIRI,

Corresponding Author: Satoshi IEIRI, M.D., and Ph.D.

Department of Pediatric Surgery, Research Field in Medical and Health Sciences,

Medical and Dental Area, Research and Education Assembly, Kagoshima University,

8-35-1, Sakuragaoka

, Kagoshima City, 890-8520, JAPAN

Tel: +81-99-275-5444

Fax: +81-99-275-2628

E-mail: sieiri@m.kufm.kagoshima-u.ac.jp

Abstract

Purpose: Treatment for a large abdominal wall defect remains challenging. The aim of this study was to optimize tissue engineering therapy of muscle constructs using a rat model.

Methods: Experimental abdominal wall defects were created in Wister rats. The animal model was divided into 3 groups: collagen sponge (CS), hybrid scaffold (HS) and hybrid scaffold containing bone marrow liquid (HSBM). Hybrid scaffolds comprised collagen sponge and poly L-lactide (PLLA) sheets. Abdominal wall defects were covered by three kinds of sheets. Thereafter, the bone marrow liquid was spread onto the sheets. Rats were sacrificed at 4, 8, and 16 weeks. Pathological examinations were performed using hematoxylin-eosin and desmin antibody staining.

Results: The CS group showed abdominal hernia, whereas the HS and HSBM groups did not. Vascular formation was confirmed in all groups. Muscle tissue was recognized at the marginal area of the sheet only in the HSBM group.

Conclusion: The HS and HSBM groups show a greater intensity than the CS group. Muscle tissue regeneration is solely recognized in the HSBM group. Our experimental data suggest that the triad of scaffold, cell, and growth factor is fundamental for ideal biomaterials. The HSBM may be useful for reconstruction of abdominal wall defects.

(200 Words/limit 200 words)

Key Words: omphalocele, gastroschisis, regenerative medicine, tissue engineering, poly L-lactide, bone marrow liquid

Introduction

The treatment of giant omphaloceles and severe gastroschisis remains a challenge for pediatric surgeons because of the underdeveloped abdominal cavity and large abdominal wall defect. Several techniques have been described to repair omphaloceles and gastroschisis.

The component separation technique (CST), which was first described in 1990 for midline abdominal wall reconstruction, is a type of rectus abdominis skeletal muscle advancement flap that allows reconstruction of such large ventral defects [1]. The advantages of CST are as follows: (1) functional and structural integrity of the abdominal wall, (2) stable soft tissue coverage, (3) improved cosmesis [1]. Despite of the advantages of autografts, including no risk of rejection, some problems must be solved. Common complications are surgical site infections, seroma, hematoma, and skin flap necrosis [2]. Moreover, wound complications occur in 33% of patients and the recurrence rate of herniation was 30% of adult cases [2].

Abdominal wall transplantation after intestinal transplantation was first described in 2003 by Levi et al. [3]. However, lifelong immunosuppressive therapy is necessary to prevent rejection [4].

On the other hand, the use of prosthetic material dramatically reduces the incidence of recurrence associated with ventral hernia repair in adult cases. Synthetic materials are available as both absorbable and non-absorbable mesh. Compared with biological grafts, the advantages of synthetic materials include greater availability (does not require harvesting) and lower material cost. Polypropylene is the most commonly used material. However, permanent non-absorbable prostheses occasionally cause foreign body reactions, resulting in significant adhesion formation and refractory infection. In pediatric patients, these non-absorbable materials are unfavorable for their growth.

Currently available biosynthetic grafts include human cadaveric dermis (allografts)

and porcine dermal and submucosal sources (xenografts). All these grafts are composed of non-cellular collagen scaffolds that are resistant to infection [5]. Biosynthetic grafts have a favorable advantage in contaminated wounds, such as traumatic case [5]. However, the availability of cadavers as a source of human dermis is limited. Xenografts are more readily available compared with allografts. However, the disadvantage of allografts and xenografts is the induction of severe immune responses [6].

Thus, there is currently no standard treatment for large abdominal wall defects and giant abdominal incisional hernia. The tissue engineering approach and regenerative medicine are promising strategies to solve this problem. A combination of scaffolds, cultured cells, and growth factors has been used in tissue engineering [7, 8]. Cell sources for tissue engineering are focused on stem cells including embryonic stem (ES) cells, bone marrow mesenchymal stem cells (BM-MSCs) and induced pluripotent stem (iPS) cell [9, 10]. However, ES cells have an ethical problem and iPS cells have the risk of tumorigenesis [10]. Such approaches remain far from clinical application. The bone marrow contains powerful multipotent progenitor cells, such as mesenchymal stem cells [11]. Bone marrow transplantation is a safe and standard treatment for leukemia and aplastic anemia. Clinical studies of cell therapy for heart failure and retinal disease have been attempted [12, 13]. At present, however, it is difficult to provide sufficient material strength for tissue regeneration.

The essential features of reconstructive materials are adequate strength, no hypersensitivity reactions, and biocompatibility to facilitate tissue ingrowth [14]. Collagen sponges are not strong, but have favorable biocompatibility. Poly L-lactide (PLLA) is not a biological material, but is safe, stable and absorbable. Therefore, we created a regeneration strategy using a collagen sponge and PLLA.

The aim of this study was to optimize tissue engineering therapy for skeletal muscle reconstruction using a rat model of abdominal wall defects.

Methods

The experimental procedures were approved by the animal care committee of Kagoshima University, Kagoshima, Japan.

Abdominal wall defect animal model

Male Wistar rats (Kyudo, Kumamoto, Japan) with a body weight of 200–220 g were used. The rats were anesthetized with an intraperitoneal injection of 100 mg/kg Ketalar@ (ketamine) (Sankyo, Tokyo, Japan) and 10 mg/kg Domitor@ (medetomidine hydrochloride) (Pfizer, Kirkland, Canada). The abdomen was shaved and cleaned with 70% alcohol. The operative procedure was performed under clean, but non-sterile conditions. In all animals, laparotomy was performed using a midline incision of 4 cm. The skin was preserved to close the wound and a round defect, measuring 2 cm in diameter, consisting of fascia, muscle, and peritoneum was created, as shown in Fig. 1a. We divided the animals into 3 groups as follows: (1) collagen sponge (CS) group, (2) hybrid scaffold (HS) group containing the CS + PLLA, and (3) hybrid scaffold + bone marrow (HSBM) group.

CS group (n=5)

The collagen sponge (KOKEN, Tokyo, Japan) was prepared for the experiment. In the CS group (n=5), the defect was repaired with only collagen sponge (CS). The CS was fixed by 10 interrupted 4–0 Maxon sutures (Covidien, Mansfield, MA, USA); the wound and the skin were closed in layers with 2–0 Dexon sutures (Sherwood Medical, St. Louis, MO, USA).

HS group (n=15)

The hybrid scaffold comprised the CS and a poly L-lactide (PLLA) sheet (COREFRONT, Tokyo, Japan). The PLLA sheet was placed on the CS and fixed using Fibrast spray@ (recombinant human basic fibroblast growth factor) (Kaken Pharmaceutical Co., Shizuoka, Japan). The abdominal wall defect was covered with the HS (Fig. 1b). The HS was fixed as in the CS group, and the skin was closed in layers (Fig. 1c).

HSBM group (n=15)

A male Wistar rat (7-week-old, body weight 200 g) was sacrificed. After extraction of the femoral bone, the bone marrow liquid was aspirated and obtained. We added normal saline solution and adjusted the final concentration to 1.0 x 10^6 cells/mL. In the HSBM group, the defect was repaired with the HS and bone marrow liquid that was sprayed and seeded onto the HS, as shown in Fig. 1d.

Tissue sampling

Abdominal wall defect rats that underwent surgical repair procedures were sacrificed. The CS group was evaluated at 4 weeks. The HS and HSBM groups were evaluated at 4, 8, 16 weeks after operation. The repaired abdominal wall was excised and sampled, including the muscle tissue.

Histological evaluation

Sampled tissues were fixed in 10% neutral buffered formalin and embedded in paraffin. Five mm thick vertical sections of implants were stained with hematoxylin and eosin (H&E) and immunohistochemical analyses were performed using a mouse-derived monoclonal antibody to desmin (DAKO, Carpinteria, CA, USA). A commercial immunohistochemical kit was used with 3, 3 diaminobenzidine tetrahydrochloride (DAB;

DAKO) as the enzyme substrate, with granular brown deposits indicating positivity.

Quantitative analysis for vascular formation

A quantitative analysis was performed using H&E staining. Vascular formation measurements were performed in 10 fields per specimen in a randomized fashion. The capillary density was measured and modified using the methods of Nowacki et al. [15]. The total number of vascular capillaries was counted in all specimens per high-power fields (40x). The capillary density was evaluated by counting the number of vessels <20 µm in diameter per high-power field (40x). Capillaries density was graded on a scale of 0-3 as follows: 0, absent; 1, low (<5 vessels); 2, moderate (5-8 vessels); and 3, and high (>8 vessels).

Statistical analysis

Statistical differences between all tested groups were evaluated using the Mann-Whitney U test. A p value < 0.05 was considered to indicate statistical significance.

Results

All animals in the three groups were anesthetized and underwent surgical procedures without complications. The rats recovered from the surgical interventions and no mortality was observed.

CS group

All rats in the CS group developed an abdominal wall hernia early after operation (Fig. 2a). An obvious intestinal prolapse was recognized in the subcutaneous space (Fig. 2b). Therefore, a long-term survival study in the CS group was difficult to carry out, and evaluations were performed after only 4 weeks. Furthermore, the CS was absorbed rapidly *in vivo*, and therefore a histological examination of the CS group was impossible.

HS and HSBM groups

No abdominal hernia was recognized in any animals repaired with the HS and HSBM (Fig. 2c). No incision hernia was noted in the intraoperative findings. An intestinal prolapse was not recognized in the subcutaneous space (Fig. 2d). In sampling the abdominal wall, including the implant, mild adhesions between the implants and omentum were observed. Adhesiolysis was easy to perform. Macroscopic differences were not observed in both groups.

Evaluation of vascular formation

Histologically, H&E staining revealed clearly visible vessels containing red blood cells in all animals in the HS and HSBM groups (Fig. 3). The capillary density was evaluated and graded using the method of Nowacki et al.

Table 1 shows the capillary density expressed as the Mean ± S.E.M. At 4 weeks, the

capillary density score of the HSBM group (2.50 ± 0.10) was significantly higher than that of the HS group (1.52 ± 0.13) (p=0.0000198). At 8 weeks, the capillary density score of the HSBM group (2.76 ± 0.07) was significantly higher than that of the HS group (2.16 ± 0.12) (p=0.000186). At 16 weeks, the capillary density score of the HSBM group (2.34 ± 0.12) was significantly higher than that of the HS group (1.20 ± 0.08) (p=0.000000142).

Muscle tissue regeneration

Fig. 4 shows a representative image of H&E staining and desmin staining of the HSBM group at 16 weeks to evaluate skeletal muscle tissue regeneration. At the center of the implant, skeletal muscle cells were not recognized in either staining method. Regarding the border lesion, skeletal muscle cell replacement was apparent (Fig. 4). Skeletal muscle cell replacement was observed at 40% at 4 weeks, 20% at 8 weeks and 20% in 16 weeks, respectively (data not shown).

Discussion

In this study, we investigated the ability of tissue engineering therapy for abdominal wall defects using a rat model.

The major findings of this study were as follows: (1) a hybrid scaffold using a CS and PLLA was feasible for the abdominal hernia; (2) vascular formation in the HSBM group was clearly recognized compared with the HS group; and (3) skeletal muscle cell replacement in the implant was only recognized in the HSBM group.

A combination of scaffolds, cultured cells, and growth factors has been used in successful tissue regeneration [8]. The ideal scaffold should be biocompatible, highly porous, and biodegradable [16]. Collagen is the main component of connective tissues and the most abundant protein in the human body. Collagen is a biocompatible and biodegradable resource. Thus, collagen is an excellent and essential material for creating scaffolds for tissue engineering [16]. Collagen also has a high affinity of cell adherence and growth-stimulating effects. However, a major disadvantage of collagen is it's readily absorbable *in vivo*. The resolution time of collagen *in vivo* is less than 2 weeks. Thus, collagen alone is not appropriate for tissue engineering due to its resolution ability.

PLLA is an innovative material that has been developed for a wide range of applications because of its biodegradability [17]. The resolution time of PLLA is more than 2 years. PLLA is a slowly degraded biopolymer that maintains the mechanical strength of grafts for a prolonged time. PLLA also allows gradual replacement of synthetic scaffolds by native matrix over time [18].

We herein used basic fibroblast growth factor (bFGF) to prepare a PLLA-collagen hybrid scaffold. Recently, it has become clear in *in vitro* experiments that bFGF exhibits a stimulation effect on the proliferation, chemotaxis, and differentiation of various types of cells, such as vascular endothelium cells, vascular smooth muscle cells, and epithelial cells, in

addition to a proliferation effect on fibroblasts. Furthermore, an *in vivo* study clearly demonstrated that bFGF had a strong neovascularization effect [19]. In this study, bFGF induced neovascularization into the implant. Using a hybrid scaffold, vascular formation was strongly induced in the HSBM group by supplementation with bone marrow liquid and bFGF. In addition to bFGF, bone marrow liquid may play an effective and important role in vascular formation. The blood flow to the surrounding tissue could be maintained by this neovascularization.

Muscle regeneration occurs in the three following steps: (1) initiations of the inflammatory response; (2) activation, differentiation, and fusion of satellite cells; and (3) maturation and remodeling of newly formed myofibers [20]. Skeletal muscle satellite cells were first described and named according to their anatomic location between the myofiber plasma and basement membranes. Satellite cell implantation has been a promising method for skeletal muscle regeneration. However, migration of satellite cells inhibited successful results in *in vivo* experiments [21].

On the other hand, the bone marrow contains a population of rare progenitor cells, known as mesenchymal stem cells (MSC), that are capable of replication as undifferentiated cells or differentiation into bone, cartilage, fat, muscle, tendon or marrow stroma. In this study, we used bone marrow liquid. The bone marrow liquid has the potential to induce cells to differentiate into skeletal muscle cells, fascia, peritoneum or fat tissue. However, skeletal muscle regeneration was not recognized in our short-term study. Therefore, future long-term studies are necessary.

In conclusion, our experimental data suggested that the triad of scaffold, cell, and growth factor is fundamental for ideal biomaterials. The HSBM may be useful for reconstruction of abdominal wall defects. However, an experimental long-term survival study is necessary before clinical trials.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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Figure/Table Legends

Figure 1

Rat model of abdominal wall defects.

a, A round defect of the abdominal wall.

b, An abdominal wall defect covered with the HS.

c, The HS fixed by 10 interrupted 4–0 Maxon sutures.

d, The HSBM with the HS and bone marrow liquid.

Figure 2

An abdominal wall hernia was observed in the CS group. (a, b)

No abdominal hernia was observed in the HS and HSBM group. (c, d)

Figure 3

Histological examinations of the HS and HSBM groups at 4, 8 and 16 weeks after operation (light microscopy, magnification 40x, H&E staining).

Figure 4

Histological examinations of the HSBM group 16 weeks after operation (light microscopy, magnification 4x, H&E and desmin staining).

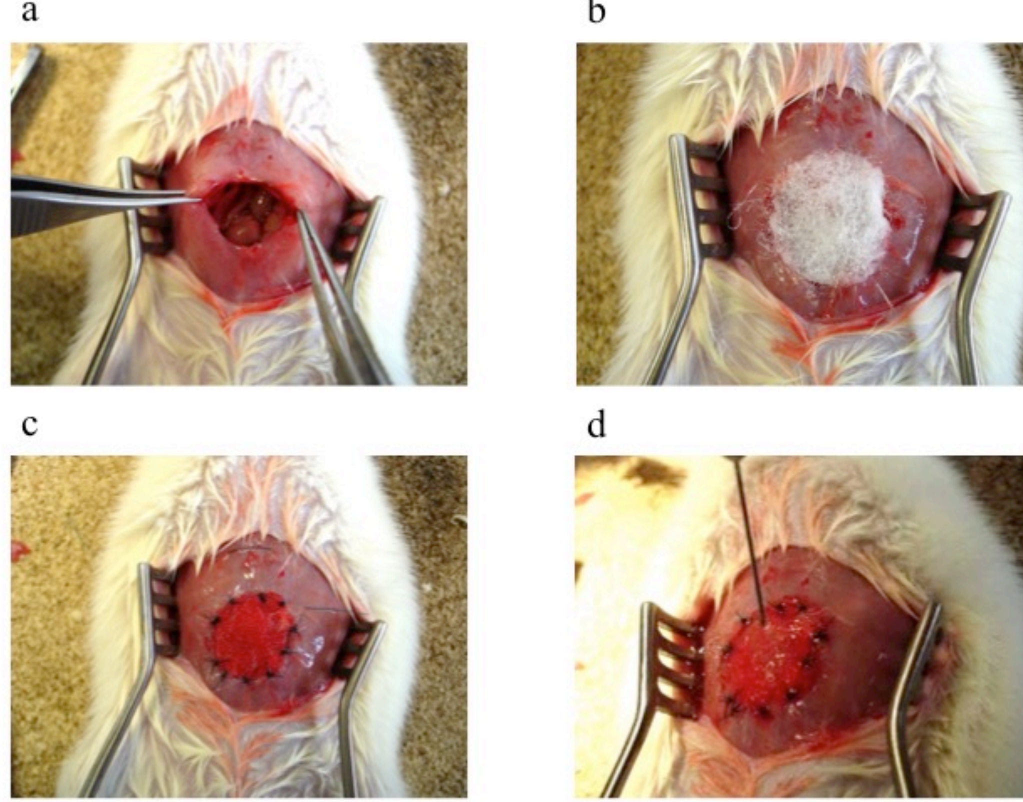
Table 1

Average capillary densities in the HS and HSBM groups.

Data are expressed as the Mean ± S.E.M.

Statistical analyses were performed at 4, 8 and 16 weeks, respectively.

Fig.1



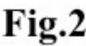


Fig.2

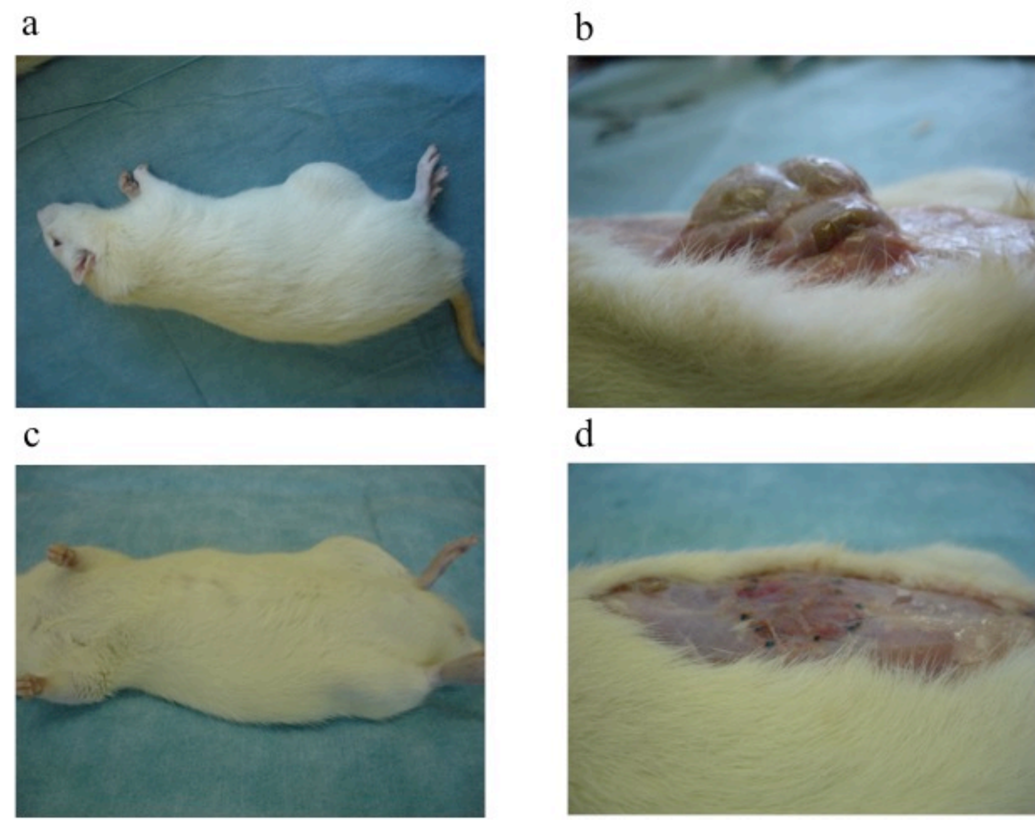


Fig.3

	4w	8w	16w
HS			
HSBM			

Fig.4

	Native muscle	Border lesion	Central lesion
H&E			
desmin			

Table 1

	Mean ± standard error		
	4 weeks	**8 weeks**	**16 weeks**
HS (n=5)	**1.52 ± 0.13**	**2.16 ± 0.12**	**1.2 ± 0.08**
HSBM (n=5)	**2.5 ± 0.10**	**2.76 ± 0.07**	**2.34 ± 0.12**
***p*-value**	**0.0000198**	**0.000186**	**0.000000142**