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REVIEW ARTICLE

Regenerative medicine using stem cells from human exfoliated deciduous teeth (SHED): a promising new treatment in pediatric surgery

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Abstract
Stem cells from human exfoliated deciduous teeth (SHEDs), being a type of mesenchymal stem cell, are an ideal cell source for regenerative medicine. They have minimal risk of oncogenesis, high proliferative capacity, high multipotency, and immunosuppressive ability. Stem cell transplantation using SHED has been found to have an anti-fibrotic effect on liver fibrosis in mice. SHED transplantation and the bio 3D printer, which can create scaffold-free 3-D images of the liver and diaphragm, provide a new innovative treatment modality for intractable pediatric surgical diseases such as biliary atresia and diaphragmatic hernia.

Keywords Mesenchymal stem cell · Dental pulp · Primary teeth · Biliary atresia · Diaphragm

Introduction

The clinical outcome of pediatric surgical disease has improved dramatically in the 54 years since the inception of the Japanese Society of Pediatric Surgeons. Now, the mortality rate associated with neonatal surgical disease is lower than 10% [1]. In fact, the overall neonatal mortality rate in Japan is the lowest in the world; however, there are still some diseases associated with high mortality or poor quality of life, despite advances in surgical treatments.

The survival rate associated with biliary atresia has improved dramatically with living-donor liver transplantation (LDLT). However, the shortage of donors and long-term complications after transplantation remain problems. Another congenital anomaly, large congenital diaphragmatic hernia, results in a large defect in the diaphragm. Both diseases have been designated as intractable and the medical costs of these patients are almost covered completely by the Japanese government. Thus, there is an urgent need for the development of new radical therapy.

Stem cells from human exfoliated deciduous teeth (SHED)

MSCs exhibit self-renewal and multipotency into a variety of mature cells and have been identified in many human tissues, including bone marrow, adipose tissue, umbilical cord blood, amniotic fluid, and dental pulp tissue. Recent studies are also being conducted to evaluate the immunomodulatory effects of MSCs, which are considered a feasible cell source for tissue engineering and regenerative medicine.

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SHEDs have been discovered in the remnant dental pulp tissue of human exfoliated deciduous teeth and share some characteristics of MSCs, including fibroblastic features, clonogenicity, cell surface antigen expression, cell proliferative capacity, and multidifferentiation potency [2] (Fig. 1). SHEDs also modulate regulatory T cells (Tregs) and dendritic cells, and have shown a better inhibiting effect in reducing interleukin-17 levels than bone marrow mesenchymal stem cells (BMMSCs) [3] (Fig. 2). Recent studies have evaluated the latent potential of SHEDs in tissue engineering for bone regeneration and cell-based therapy for a variety of refractory systemic diseases, including hypoxic-ischemic brain injury [4], systemic lupus erythematous [3], ulcerative colitis [5], spinal cord injury, Parkinson's disease, and diabetes [5]. Furthermore, exosomes derived from human dental pulp-derived stem cells are considered to be therapeutic [5].

There is accumulating evidence that a variety of human MSCs are capable of differentiating into hepatocyte-like cells in vivo in animal models of hepatic failure. Advanced tissue engineering techniques accelerate the trans-differentiation ability of human MSCs into hepatocytes. In comparison with other human tissues, exfoliated deciduous teeth offer the distinct advantages of less ethical controversy, a readily accessible source, easy and minimally invasive collection, and retention of high stem cell potential such as cell proliferation, multipotency, and immunomodulatory functions [6], even after cryopreservation (Fig. 3) [7]. Several companies have been creating a SHED bank for allogenic cell therapy, as well as autologous cell therapy. Thus, exfoliated deciduous teeth might be a feasible cell source for MSC-based therapy.

Current status of pediatric liver transplantation in Japan

Liver transplantation is established as an effective innovative treatment for end-stage liver cirrhosis, acute irreversible liver failure, metabolic disease, and unresectable liver tumors. Because of the laws and cultural traditions of Japan,

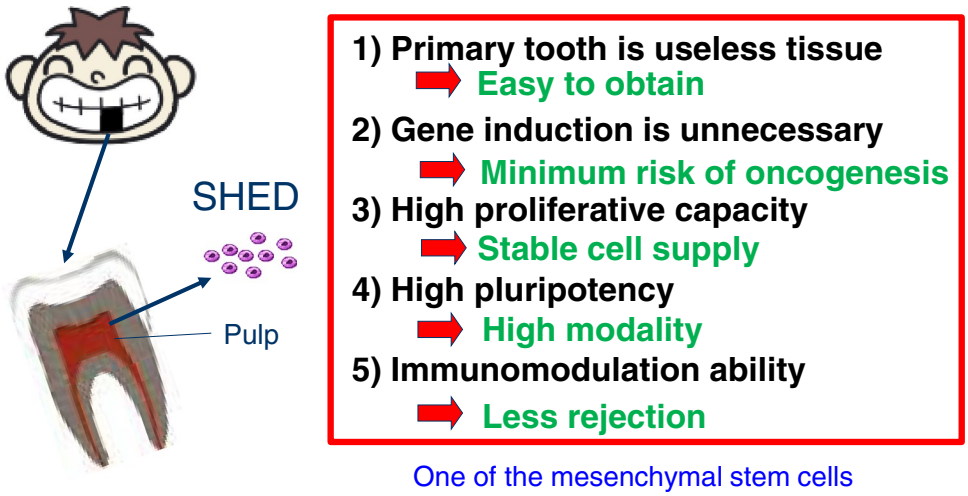

Fig. 1 Characteristics of stem cells from human exfoliated deciduous teeth (SHEDs)

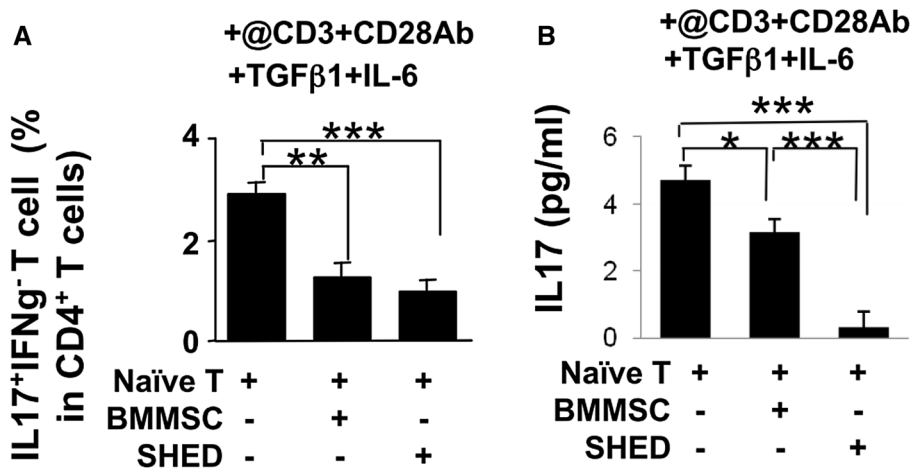

Fig. 2 Under the anti-CD3 and CD28 antibody with TGFβ1 and IL-2 stimulation, stem cells from human exfoliated deciduous teeth (SHEDs) showed a significantly greater effect in reducing the Th17 cell levels than bone marrow mesenchymal stem cells (BMMSCs) (**a**). However, SHEDs had a significantly greater capacity to inhibit IL17 levels than BMMSCs (**b**) (modified from reference [3])

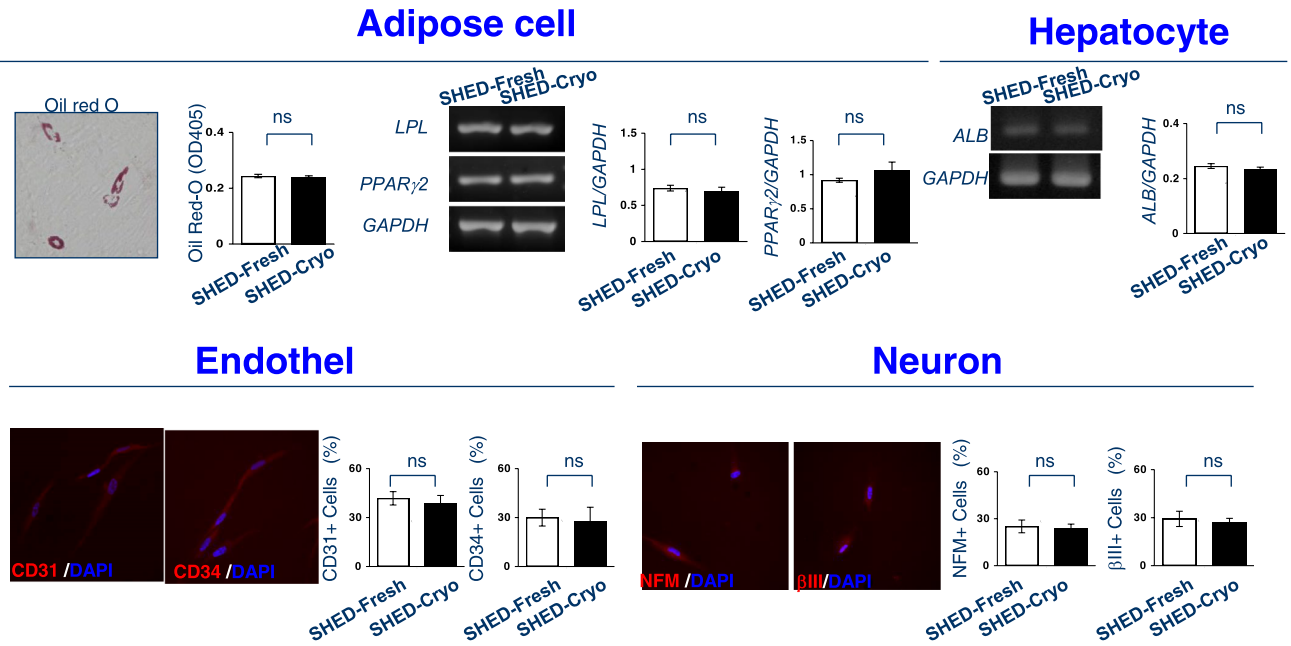

Fig. 3 Pluripotentiality of stem cells from human exfoliated deciduous teeth (SHEDs) after 2 years of frozen preservation. (modified from reference [7])

the number of LDLTs being performed has increased dramatically in this country. LDLT is suitable for pediatric liver transplantation, because the left lobe or lateral segment provides the graft volume needed, and this type of transplantation can be done electively.

The universal HBV vaccination, introduced in Japan in 2016, and the development of the oral antiviral agent for HCV will result in fewer adult liver transplantations for liver cirrhosis in the future. Conversely, the number of pediatric liver transplantations is unlikely to change, because most of the primary diseases necessitating pediatric liver transplantation are intractable. Thus, for the foreseeable future, pediatric liver transplantation will be necessary.

Although the outcome of pediatric liver transplantation is generally better than that of adult liver transplantation, there are several long-term complications, including portal vein thrombosis and chronic rejection, as well as the adverse effects of immunosuppressive therapy such as renal dysfunction, de novo malignant tumors, hypertension, and diabetes mellitus. Moreover, pediatric patients must continue to take immunosuppressive therapy forever. This reinforces the need for a safe and effective treatment alternative to pediatric liver transplantation.

Hepatocyte transplantation

There have been several reports about mature hepatocyte transplantation in clinical trials [8]. However, its effect lasts for about 3 months and it works only as bridge to liver transplantation. Because of their potential to differentiate into a variety of mature cells, stem cells are considered a feasible cell source for liver regeneration. Stem cells from human exfoliated deciduous teeth (SHED) exhibit hepatogenic capability in vitro.

We investigated the in vivo capabilities of homing and hepatocyte differentiation, and their therapeutic efficacy for liver disorders in a mouse model of carbon tetrachloride (CCl_4)-induced liver fibrosis [9]. We transplanted SHEDs into the CCl_4-induced liver fibrosis model mice, through the spleen, and analyzed the in vivo homing and therapeutic effects by optical, biochemical, histological, immunological and molecular biological assays (Fig. 4). We then sorted human leukocyte antigen-ABC (HLA-ABC)-positive cells from primary CCl_4-damaged recipient livers and analyzed their fusogenicity and hepatic characteristics by flow cytometric, genomic DNA, and hepatocyte-specific gene assays. Furthermore, we examined the treatment effects of HLA-positive cells on hepatic dysfunction by a secondary transplantation into the CCl_4-treated mice [9].

The transplanted SHEDs homed to the recipient livers and expressed HLA-ABC, human hepatocyte-specific antigen hepatocyte paraffin 1, and human albumin. SHED transplantation improved liver dysfunction remarkably, with anti-fibrotic and anti-inflammatory effects in the recipient livers. SHED-derived HLA-ABC-positive cells that were sorted from the primary recipient liver tissues with CCl_4 damage did not fuse with the host mouse liver cells. Sorted HLA-positive cells not only expressed human hepatocyte-specific genes, including albumin, cytochrome P450 1A1, fumarylacetoacetase, tyrosine aminotransferase, uridine

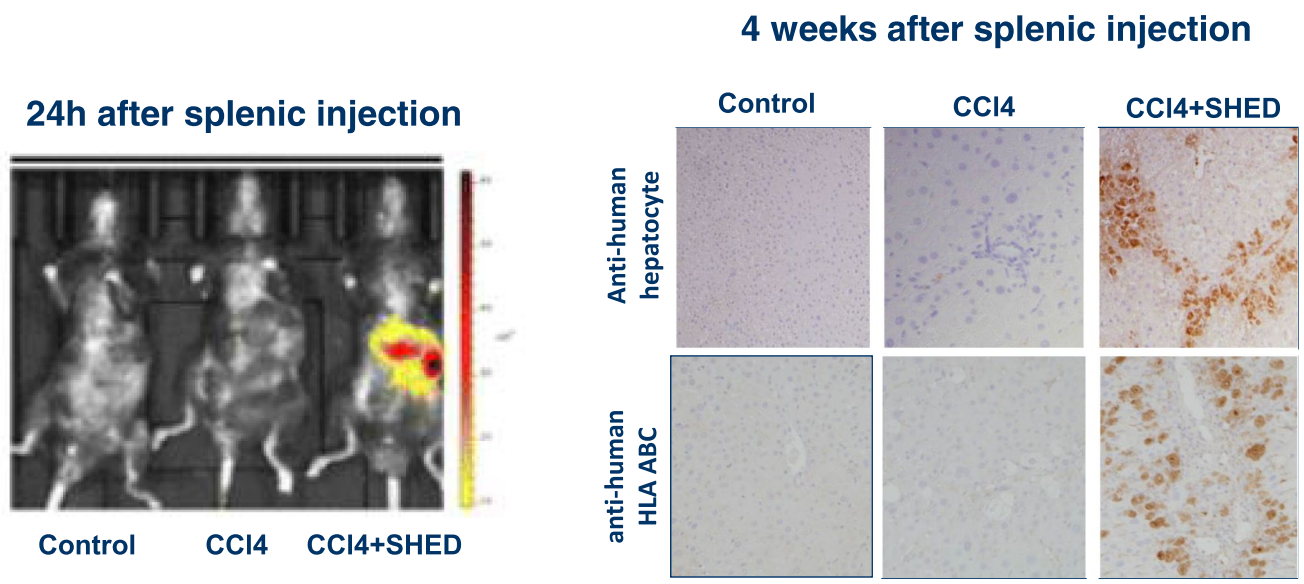

Fig. 4 The liver-homing capacity of stem cells from human exfoliated deciduous teeth (SHEDs) after splenic injection. (modified from reference [9])

5′-diphospho-glucuronosyltransferase, transferrin and transthyretin, but they also secreted human albumin, urea, and blood urea nitrogen. SHED-derived HLA-ABC-positive cells were also transplanted secondarily into CCl_4-treated mice. The donor cells homed into secondary recipient livers and expressed hepatocyte paraffin 1 and human albumin, as well as HLA-ABC. The secondary transplantation improved liver dysfunction in the secondary recipients [9].

This study demonstrates that transplanted SHEDs improved hepatic dysfunction and directly transformed into hepatocytes without cell fusion in CCl_4-treated mice, suggesting that SHEDs may provide a feasible cell source for liver regeneration.

Creation of a mini-liver

Cell-based therapy has been proposed as an alternative to orthotopic liver transplantation. The novel transplantation of an in vitro-generated liver bud might have therapeutic potential. In vivo and ex vivo methods of growing a liver bud are essential for paving the way for the clinical translation of liver bud transplantation. Takebe et al. co-cultured liver-like cells derived from iPS, endothelial cells, and MSC. As a result, they created a "liver bud" and successfully transplanted it heterotopically [10]. Sakai et al. created a "liver sheet" composed of hepatocytes and fibroblasts, and successfully transplanted it subcutaneously [11]. We reported a novel transplantation method for liver buds that are grown in vivo, involving orthotopic transplantation onto the transected parenchyma of the liver. This achieved long engraftment and better growth than heterotopic transplantation [12].

Creation of large liver

For the radical treatment of liver cirrhosis, at least 40% of the standard liver volume is required. One method involves the creation of a decellularized liver scaffold, and this scaffold is recellularized by iPS cells [13]. We are developing a method of rapidly fabricating scalable liver-like tissue by fusing hundreds of liver bud-like spheroids using a 3D bioprinter (Fig. 5). This system fixes the shape of the 3D tissue with the needle array system, enabling the fabrication of elaborate geometry and the immediate execution of culture circulation after 3D printing, thereby avoiding an ischemic environment ex vivo. The ex vivo fabricated human liver-like tissue exhibited self-tissue organization with an endothelial network and biliary duct formation (Fig. 6), also with engraftment on the liver of nude rats. These achievements show conclusively both in vivo and ex vivo methods of growing in vitro-generated liver buds [12]. These methods provide a new approach for in vitro-generated liver organoids transplantation. The creation of a scaffold-free 3D organ can be achieved by this unique method (Fig. 7).

Congenital diaphragmatic hernia

The need for alternatives to native diaphragmatic tissue is critical for this type of pediatric surgery. The clinical efficacy of mesh patches is limited by complications associated with residual foreign material and by hernia recurrence. Diaphragmatic repair using synthetic materials can induce inflammatory response, adhesions, and with less compliance than native tissue [14]. We used a novel bio-3D printer method to generate large scaffold-free tissue patches composed of human cells

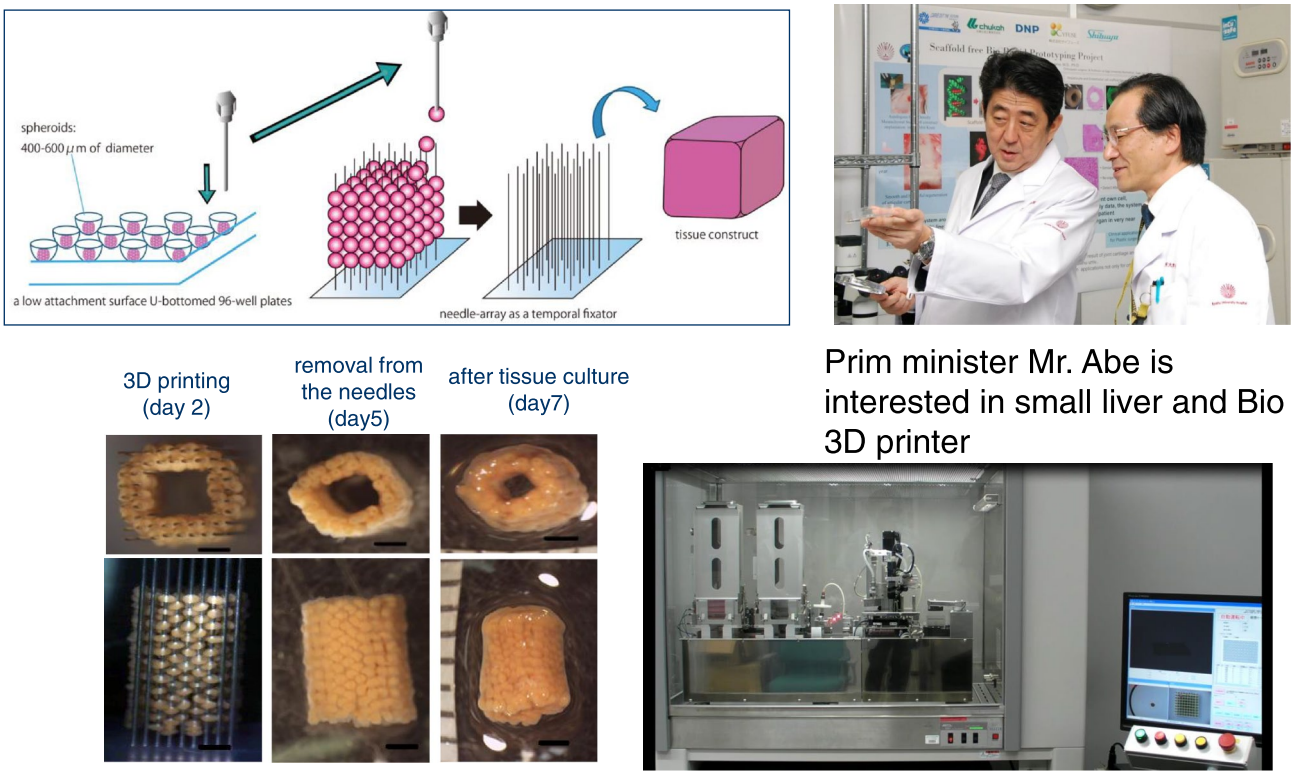

Fig. 5 Ex vivo fabrication of scalable human liver-like tissue using bio-3D printing technology

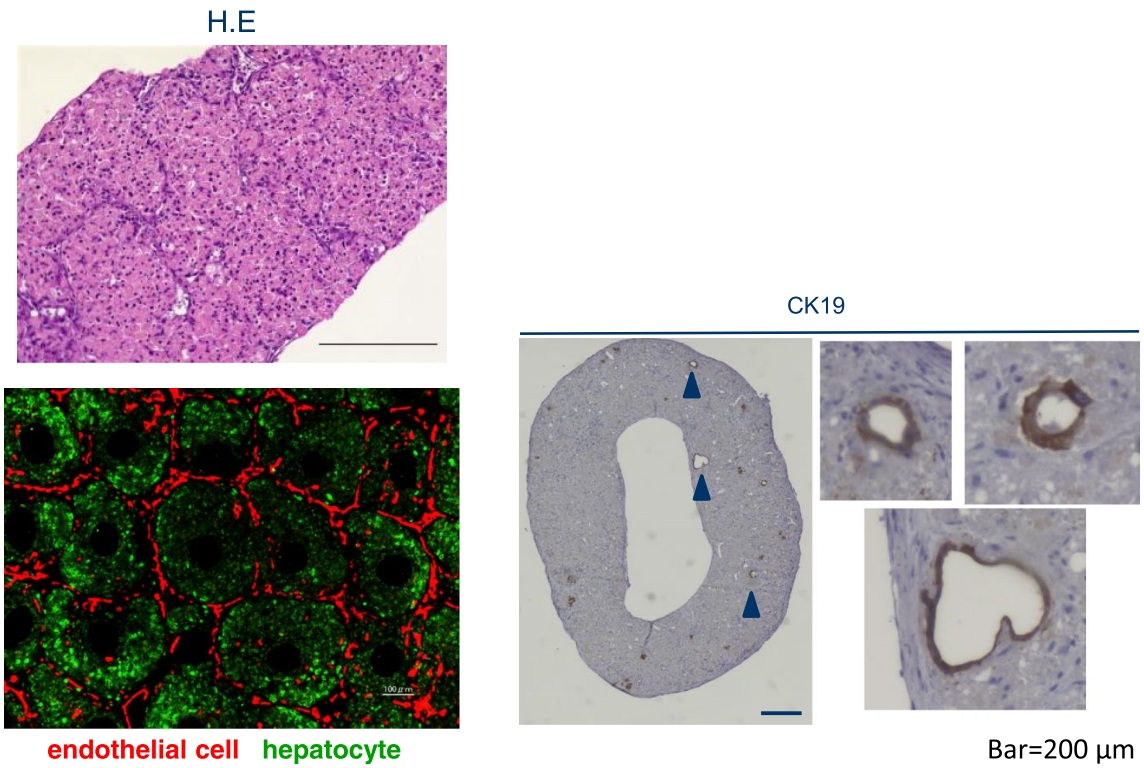

Fig. 6 An HE-stained cross-section showed viable hepatocytes and a fluorescence image of liver-like tissue showed the formation of reticular endothelial networks. Immunostaining of liver-like tissue revealed CK19-positive cells with a duct-like morphology (modified from reference [12])

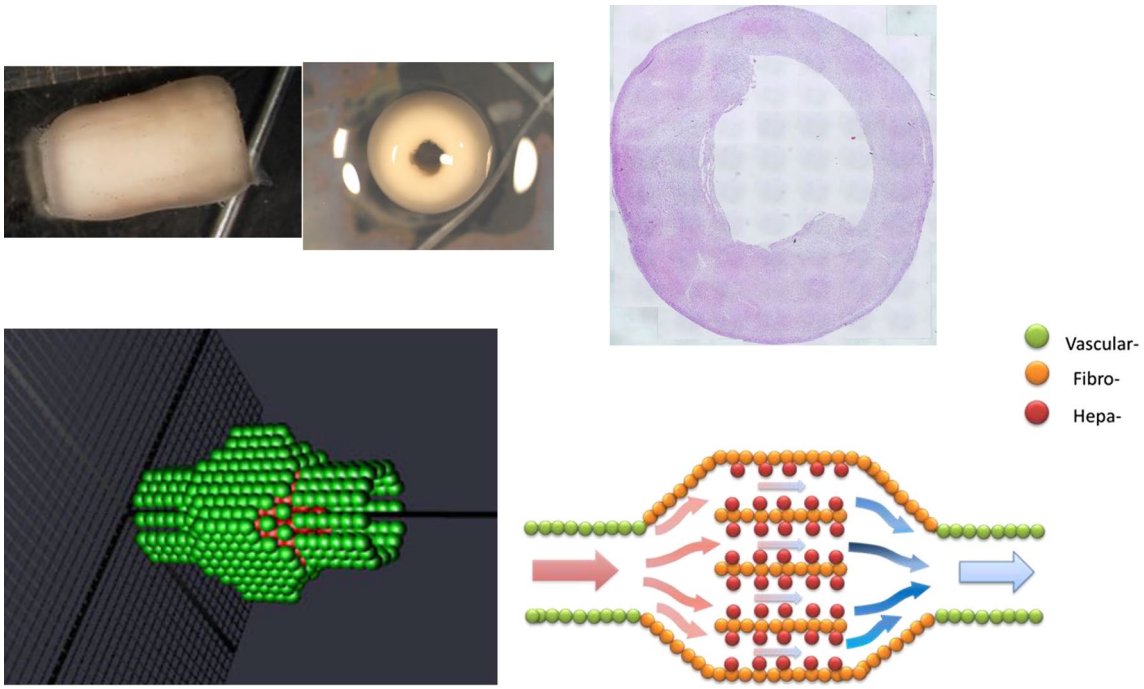

Fig. 7 Scaffold-free artificial liver with a vascular pedicle (image)

[15]. The resulting large tissue constructs had good elasticity and strength. Cellular patches were transplanted into rats with surgically created diaphragmatic defects (Fig. 8). The rats survived for over 710 days after the implantation of tissue constructs. CT confirmed complete tissue integration of the grafts during rat growth and histology revealed the regeneration of muscle structure, neovascularization, and neuronal networks within the reconstructed diaphragms. Moreover, SHEDs have multipotency, including mesenchymal lineage with myocytes, adipocytes, and chondrocytes [5], and dental pulp stem cells can repair myocardiac tissue damaged by infarction [16]. Our results demonstrate that created cellular patches are a safe

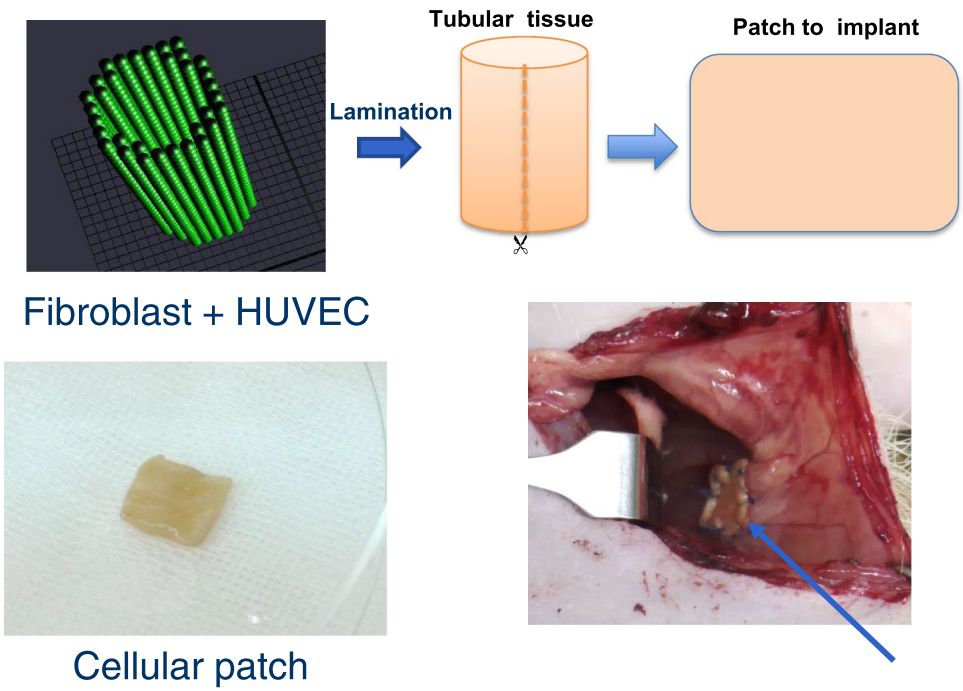

Fig. 8 Scaffold-free tissue-engineered diaphragm (modified from reference [13])

and effective therapeutic strategy for repairing diaphragmatic defects and pave the way for a clinical trial.

Clinical application of SHED therapy

There are two possibilities for the design of a clinical trial of SHED. One is as autotransplantation for diseases without genetic abnormality, such as biliary tresia and congenital diaphragmatic hernia. The other is as allotransplantation for diseases with genetic abnormality, such as metabolic disease, coagulopathy (including hemophilia A&B), and familiar extensive aganglionosis. To set up the allotransplantation program, a SHED bank must be established. As SHEDs show immunotolerance activity, allotransplantation is feasible. Looking for HLA-homo donors and creating a SHED bank will allow us to proceed with this project. If we can find HLA-homo donors with HLA-A*24:02-B*52:01-DRB1*15:02 and HLA-A*33:03-B*44:03-DRB1*13:02, then 24% of the Japanese population will be covered.

Summary and future prospects

SHEDs are an ideal cell source for regenerative medicine with minimal risk of oncogenesis, high proliferative capacity, high multipotency, and immunosuppressive ability. Stem cell transplantation using SHEDs had an anti-fibrotic effect on liver fibrosis in mice, and liver and diaphragm were able to be created using a bio 3D printer in a scaffold-free manner. SHED transplantation and the bio 3D printer provides a new innovative treatment modality for intractable pediatric surgical diseases like biliary atresia, and diaphragmatic hernia. For a clinical trial of SHEDs, there are two possible designs: autotransplantation and allotransplantation. Finding HLA-homo donors and creating a SHED bank will enable the SHED allotransplantation trial to proceed. Dental pulp stem cells can also be created from a wisdom tooth or a supernumerary adult tooth. Regenerative medicine using stem cells from dental pulp could represent a breakthrough in the surgical treatment of children as well as adults.

Compliance with ethical standards

Conflict of interest Tomoaki Taguchi, and his co-authors have no conflicts of interest to declare.

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