

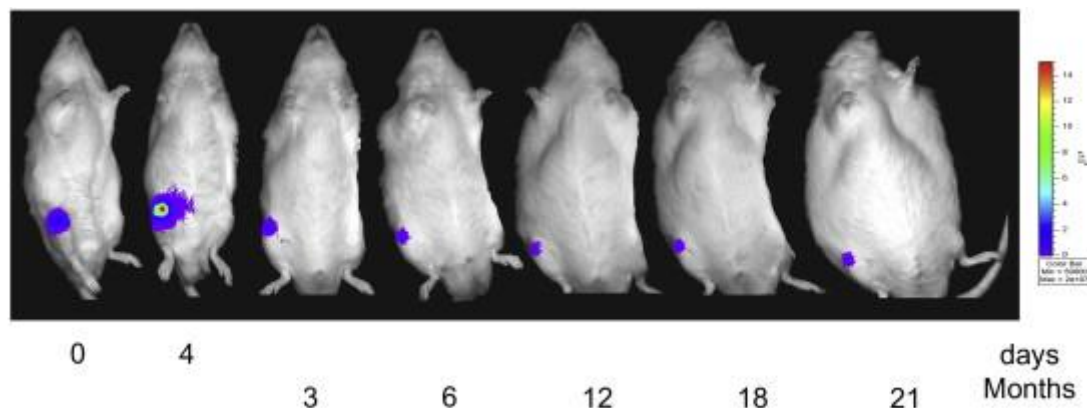
Biomaterials. 2013 [Epub ahead of print]

## In vivo cell tracking by bioluminescence imaging after transplantation of bioengineered cell sheets to the knee joint.

Takaku Y, Murai K, Ukai T, Ito S, Kokubo M, Satoh M, **Kobayashi E**, Yamato M, Okano T, Takeuchi M, Mochida J, Sato M.

### **Abstract**

In our previous studies, we have demonstrated effective regeneration of cartilage through the creation and application of layered cell sheets that combine both chondrocytes and synovial cells. In this study, we were able to demonstrate that cells derived from cell sheets can survive for long periods after transplantation into rat knee joints having osteochondral defects. We established a method for generating cell sheets from firefly luciferase-expressing chondrocytes obtained from transgenic Lewis rats, and carried out allogenic transplantation of these cell sheets into wild-type Lewis rats. We then administered luciferin and monitored the survival of the transplanted cells by using bioluminescence imaging (BLI). Our data showed that the transplanted cells survived and could be detected for more than 21 months, which was longer than expected. Furthermore, the BLI data showed that the transplanted cells remained in the knee joint and did not migrate to other parts of the body, thus confirming the safety of the cell sheets. In this study, we monitored the duration of survival of cell sheets composed of only chondrocytes, only synovial cells, or both chondrocytes and synovial cells, and found that all three types of cell sheets survived for an extended period of time.



PRS-GO 2013. [Epub ahead of print]

## **Muscle is a target for preservation in a rat limb replantation model.**

Iijima Y, Ajiki T, **Teratani T**, Hoshino Y, **Kobayashi E**.

### **Background:**

Ischemia exceeding 6 hours makes clinical limb replantation difficult and places the patient at risk of functional deficit or limb loss. We investigated the preservation of muscle function and morphology with solutions in rat hindlimb in vivo and in vitro.

### **Methods:**

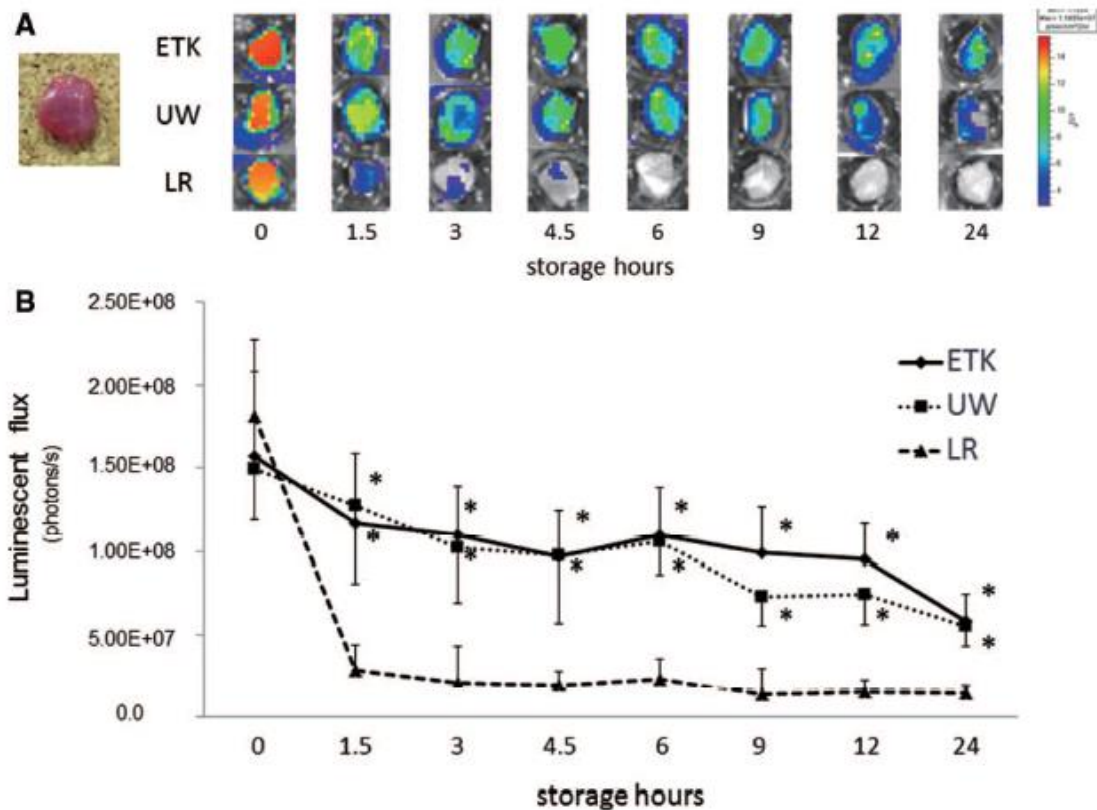
Quadriceps femoris muscles from luciferase transgenic rats were preserved for 24 hours at 4°C in extracellular-type trehalose containing Kyoto (ETK), University of Wisconsin (UW), or lactated Ringer's (LR) solution (control). Muscle luminescence was measured with a bioimaging system. Amputated limbs of Lewis rats preserved with ETK, UW, or LR for 6 or 24 hours at 4°C were transplanted orthotopically. At week 8, terminal latency and amplitude were measured in the tibialis anterior muscle. The muscles were also analyzed histologically.

### **Results:**

Isolated muscles preserved in ETK or UW had significantly higher luminescence than did muscles immersed in LR ( $P < 0.05$ ). In the 6-hour-preserved limb transplantation model, although the 3 groups had almost the same terminal latency, electrical amplitude was significantly lower in the LR group. Histologically, muscles preserved with LR showed the most atrophic changes. In the 24-hour-preserved model, the survival rate of the LR group was 37.5% in contrast to 80% in the ETK and UW groups. Electrical signals were not detected in the LR group owing to severe muscle atrophy and fibrosis. The ETK and UW groups showed good muscle function electrophysiologically.

### **Conclusions:**

Preservation solutions can protect muscle function and morphology in ischemia–reperfusion limbs and improve recipient survival rates after transplantation of long-term-preserved limbs.



Transplant Proc. 2013. [Epub ahead of print]

## Impact of ex vivo administration of mesenchymal stem cells on the function of kidney grafts from cardiac death donors in rat.

Iwai S, Sakonju I, Okano S, Teratani T, Kasahara N, Yokote S, Yokoo T, Kobayashi E.

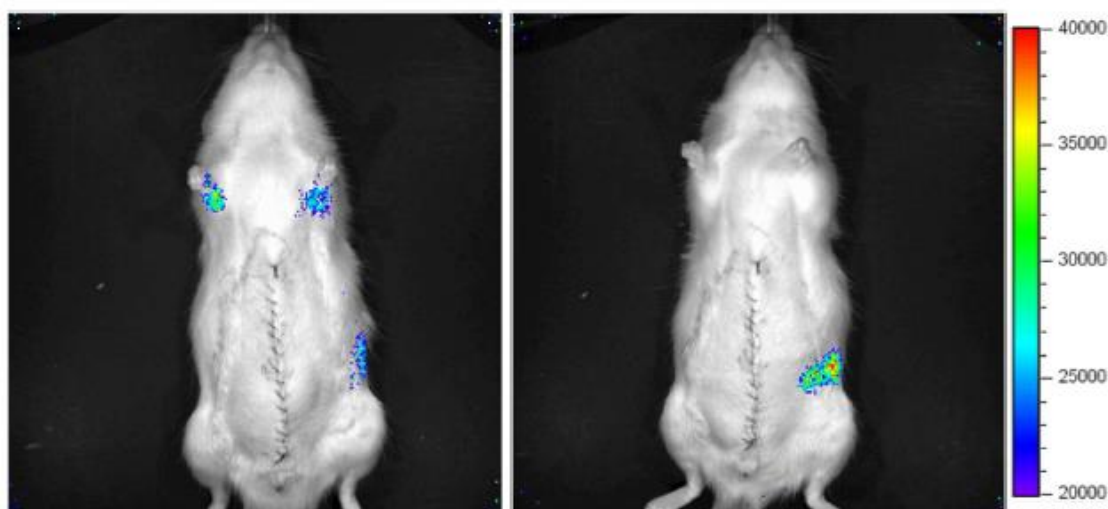
**Background:** Mesenchymal stem cells (MSCs) have been applied to the treatment of various diseases, and MSC administration in marginal donor grafts may help avoid the ischemia–reperfusion injury associated with solid organ transplants. Given the reports of side effects after intravenous MSC administration, local MSC administration to the target organ might be a better approach. We administered adipose tissue–derived MSCs (AT-MSCs) ex vivo to donor rat kidneys obtained after cardiac death (CD).

**Methods:** Using male Lewis rats (8–10 weeks), and a marginal transplant model of 1hr CD plus 1hr sub-normothermic ET-Kyoto solution preservation were conducted. AT-MSCs obtained from double-reporter (luciferase–LacZ) transgenic Lewis rats were injected either systemically (1.0x10<sup>6</sup> cells/0.5mL) to bilaterally nephrectomized recipient rats that had received a marginal kidney graft (n=6), or locally via the renal artery (500  $\mu$ L ET-Kyoto solution containing the same number of AT-MSCs) to marginal kidney grafts, which were then preserved (1hr; 22°C) before being transplanted into bilaterally nephrectomized recipient rats (n=8). Serum was collected to assess the

therapeutic effects of AT-MSc administration, and the recipients of rats surviving to Day 14 were separately evaluated histopathologically. Follow-up was by in vivo imaging and histological LacZ staining, and tumor formation was evaluated in MSC-injected rats at 3 months.

**Results:** Systemic injection of MSC did not improve recipient survival. In vivo imaging showed MSCs trapped in the lung that later became undetectable. Ex vivo injection of MSCs did show a benefit without adverse effects. At Day 14 after RTx, 75% of the rats in the AT-MSc-injected group (MSC[+]) had survived, whereas 50% of the rats in the AT-MSc-non-injected group (MSC[□]) had died. Renal function in the MSC(+) group was improved compared with that in the MSC(□) group at Day 4. LacZ staining revealed AT-MSCs attached to the renal tubules at 24hr after RTx that later became undetectable. Histopathological examination showed little difference in fibrosis between the groups at Day 14. No teratomas or other abnormalities were seen at 3 months.

**Conclusions:** Ex vivo AT-MSc injection has the potential to rescue ischemia-reperfusion injury after CD donor kidney transplantation in rats.



Transplant Proc. 2013. [Epub ahead of print]

## Evaluation of Liver Preservation Solutions by Using Rats Transgenic for Luciferase.

Doi, J, **Teratani T**, Kikuchi T, Kasahara N, Fujimoto Y, Uemoto S, **Kobayashi E**.

**Introduction.** The solution in which graft tissue is stored (that is, preservation solution) is an important component of liver transplantation technology. Its protective effect is induced by substances in the solution, including radical scavengers, buffers, and energy-giving substances. New preservation solutions have proven to be effective in preventing organ damage during cold ischemia and in extending the time limits for storage.

**Aim.** This study determines the relationship between luminescence intensity and content of adenosine triphosphate (ATP) in liver tissue and proposes a new ex-vivo screening system that uses Lewis rats transgenic for luciferase for evaluating the effectiveness of preservation solutions.

**Methods.** Samples (diameter, 2 mm) of liver were obtained from transgenic rats. The viability of these tissues after storage for as long as 6 hours in University of Wisconsin solution, extracellular trehalose solution of Kyoto, Euro-Collins solution, histidine–tryptophan–ketoflutarate solution, low potassium dextran solution, or normal saline was assessed by determining ATP content and luminescence intensity.

**Results.** Luminescence had a linear relationship ( $R = 0.88$ ) with ATP levels. Regardless of the preservation solution used, the luminescence intensities of the liver tissue chips decreased linearly with time especially through short span of time (0 to 2 hours) ( $R^2 = 0.58 - 1.0$ ). The luminescence of liver chip tissues maintained long term (2 to 6 hours) in UW solution tended to be higher than those of tissues stored in other solutions ( $p < 0.05$ , 6 hours). On the basis of luminescence intensity, EC might be preferable to the other solutions tested for ultra-short-term storage (0.5 to 2 hours).

**Conclusion.** Our model, which combines the use of the bioimaging system and Lewis rats transgenic for luciferase effectively assessed the viability of liver tissue samples. We believe that this ex vivo screening system will be an effective tool for evaluating preservation solutions for liver grafts.

Solution	Time after harvest (hour)							
	0	0.5	1	2	3	4	5	6
UW	100 ± 0	74.2 ± 2.15	61.2 ± 1.84	48.44 ± 1.61	38.09 ± 1.23	35.54 ± 1.16	32.22 ± 1.63	27.62 ± 1.48
ET-K	100 ± 0	79.67 ± 2.11	59.74 ± 0.87	40.28 ± 1.08	30.72 ± 1.13	19.59 ± 0.75	13.49 ± 0.6	8.21 ± 0.45
EC	100 ± 0	82.88 ± 2.03	67.41 ± 2.88	31.68 ± 1.55	21.29 ± 1.28	12.96 ± 0.74	9.13 ± 0.57	6.13 ± 0.43
HTK	100 ± 0	38.79 ± 1.40	17.56 ± 0.49	11.65 ± 0.6	6.37 ± 0.33	4.71 ± 0.43	2.37 ± 0.13	2.35 ± 0.14
LPDS	100 ± 0	22.67 ± 1.47	6.9 ± 0.62	2.14 ± 0.21	1.26 ± 0.11	0.77 ± 0.07	0.47 ± 0.03	0.39 ± 0.02
Saline	100 ± 0	15.69 ± 1.00	6.45 ± 0.33	2.08 ± 0.16	0.93 ± 0.06	0.56 ± 0.03	0.43 ± 0.02	0.37 ± 0.02

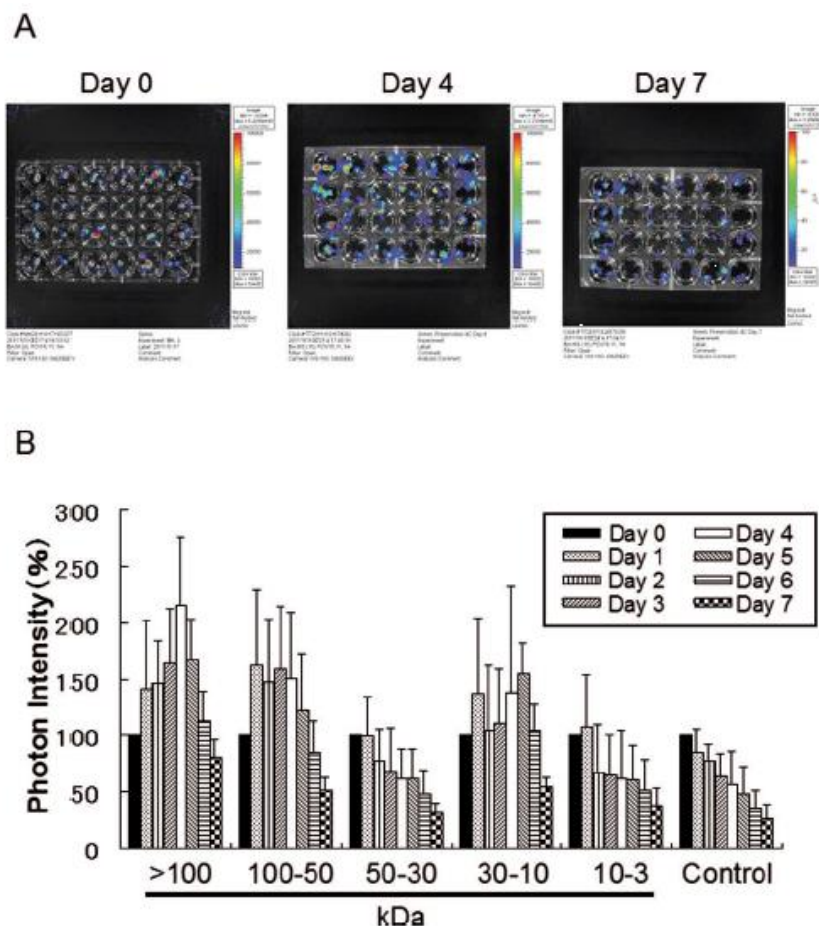
Cell Med. 5, 75-81. 2013.

Use of mesenchymal stem cell-conditioned medium to activate islets in preservation solution.

Kasahara N, Teratani T, Doi J, Iijima Y, Maeda M, Uemoto S, Fijimoto Y, Sata N, Yasuda Y, Kobayashi E.

**Abstract**

Pancreatic islet transplantation has received widespread attention as a promising treatment for type 1 diabetes. However, islets for transplantation are subject to damage from a number of sources, including ischemic injury during removal and delivery of the donor pancreas, enzymatic digestion during islet isolation, and reperfusion injury after transplantation in the recipient. Here we found that protein fractions secreted by mesenchymal stem cells (MSCs) were capable of activating preserved islets. A conditioned medium from the supernatant obtained by culturing adipose tissue MSCs (derived from wild-type Lewis rats) was prepared for 2 days in serum-free medium. Luc-Tg rat islets to which an organ preservation solution was added were then incubated at 4°C with fractions of various molecular weights prepared from the conditioned medium. Under the treatment with some of the fractions, by 4 days the relative luminescence intensities (representative of the ATP levels of the cold preserved islets) had increased to over 150% of their initial values. Our novel system may be able to restore isolated islets to the condition they were in before transport, culture, and transplantation.



Transplant Proc. 2013 Jul-Aug;45(6):2486-90.

## Luminescence-based assay to screen preservation solutions for optimal ability to maintain viability of rat intestinal grafts.

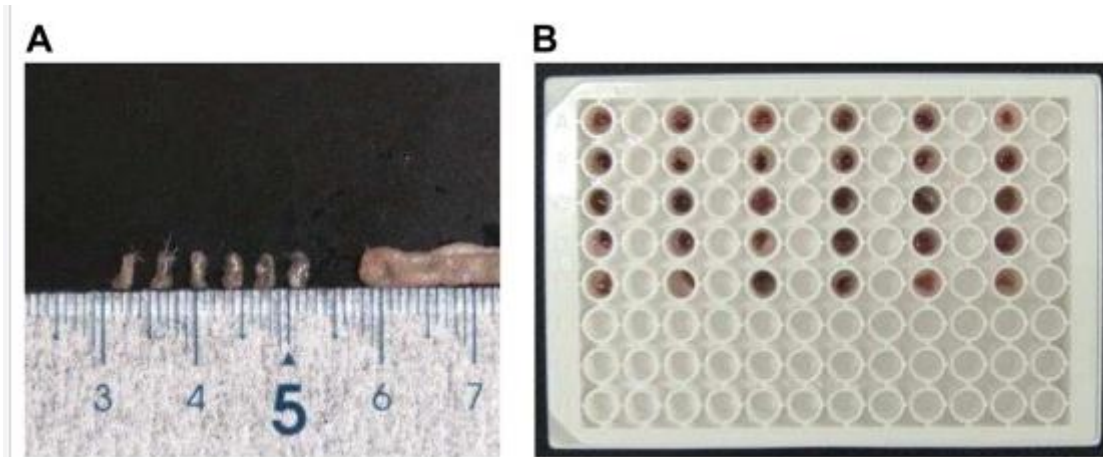
Kasahara N, Kikuchi T, Doi J, **Teratani T**, **Fujimoto Y**, Uemoto S, Yasuda Y, **Kobayashi E**.

**BACKGROUND:** Segmental intestinal transplantations from living, genetically related donors provide advantages compared with those from cadaveric subjects. However, successful preservation during ischemic cold storage is critical for living donor grafts. Thus, the development of preservation solutions that maintain graft viability is essential for success. Herein we have reported application of a cell-based viability assay in multiwell plates to assess the effectiveness of various solutions to preserve intestinal grafts.

**METHODS:** Freshly isolated intestinal chips from luciferase transgenic rats were placed in 96-well tissue culture plates for incubation at 4°C for 24 hours in various preservation solutions: ET-Kyoto (ET-K), University of Wisconsin (UW) solution, Euro-Collins (EC) solution, histidine-tryptophan-ketoglutarate (HTK) solution, lactated Ringer's (LR) solution, or saline.

**RESULTS:** As indicated by a higher level of luminescence, intestinal chips preserved in UW, HTK, or ET-K solution contained more viable cells, than those preserved in EC, LR, or saline solution. After exposure to the preservation solutions for 1 hour, the mucosal layer chips showed lower cell viability than the muscle layer chips.

**CONCLUSION:** Our data demonstrated that ET-K and UW solutions used together with intestinal chips of Luciferase transgenic rat and in vivo imaging provided optimal viability during ischemic cold storage prior to transplantation. Further development of preservation conditions to minimize the loss of viability of intestinal grafts before clinical transplantation is essential to improve outcomes.



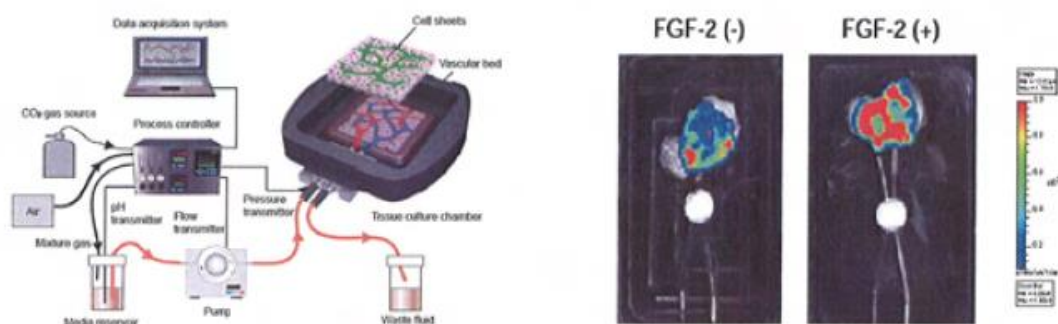
Nat Commun. 2013;4:1399.

## In vitro fabrication of functional three-dimensional tissues with perfusable blood vessels.

Sekine H, Shimizu T, Sakaguchi K, Dobashi I, Wada M, Yamato M, **Kobayashi E**, Umezu M, Okano T.

### Abstract

In vitro fabrication of functional vascularized three-dimensional tissues has been a long-standing objective in the field of tissue engineering. Here we report a technique to engineer cardiac tissues with perfusable blood vessels in vitro. Using resected tissue with a connectable artery and vein as a vascular bed, we overlay triple-layer cardiac cell sheets produced from coculture with endothelial cells, and support the tissue construct with media perfused in a bioreactor. We show that endothelial cells connect to capillaries in the vascular bed and form tubular lumens, creating in vitro perfusable blood vessels in the cardiac cell sheets. Thicker engineered tissues can be produced in vitro by overlaying additional triple-layer cell sheets. The vascularized cardiac tissues beat and can be transplanted with blood vessel anastomoses. This technique may create new opportunities for in vitro tissue engineering and has potential therapeutic applications.



Heart Asia. 2013 Jan 17;5(1):7-14.

## A luminance-based heart chip assay for assessing the efficacy of graft preservation solutions in heart transplantation in rats.

Maeda M, Kasahara N, Doi J, Iijima Y, Kikuchi T, **Teratani T**, **Kobayashi E**.

### ABSTRACT

**Objective:** We developed a novel luciferase-based viability assay for assessing the viability of hearts preserved in different solutions. We examined whether this in vitro system could predict heart damage and survival after transplantation in rats.

**Design:** By our novel system, preserved heart viability evaluation and transplanted heart-graft functional research study.



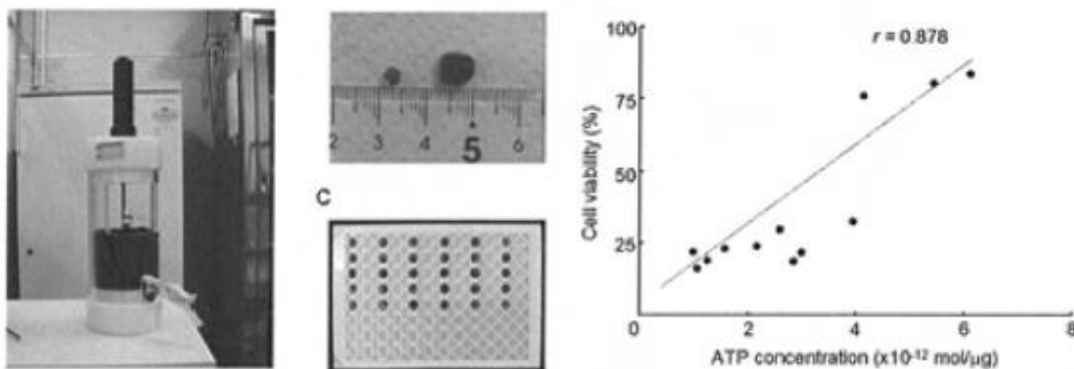
**Setting:** University basic science laboratory.

**Interventions:** Isolated Luciferase-transgenic Lewis (LEW) rat cardiac-tissue-chips were plated on 96-well tissue-culture plates and incubated in preservation solutions at 4°C. Viability was measured as photon intensity by using a bio-imaging system. Heart-grafts preserved in University of Wisconsin (UW), extracellular-trehalose-Kyoto (ETK), Euro-Collins (EC), histidin-tryptophan-ketoglutarat solution (HTK), lactated Ringer's (LR) or normal saline solution were transplanted cervically by using a cuff-technique or into the abdomens of syngeneic wild-type LEW rats by using conventional microsurgical suture techniques.

**Main outcome measures:** Imaging an evaluation of preservation heart-graft and functional analysis.

**Results:** Cardiac-tissue-chips preserved with UW, HTK or ETK solution gave higher luminance than those preserved with EC, LR or normal saline ( $p < 0.03$ ). After 24 h of preservation of hearts in each solution at 4°C, the beating of the isolated hearts was evaluated. The success rate, evaluation of beating, of cervical heart transplants using UW and ETK solution exceeded 70%, but those using other preservation solutions were lower (UW: 100%, ETK: 75%, EC: 42.86%, HTK: 14.29%, normal saline: 0%). Histological analysis of cervical heart-grafts after 3 h preservation by myeloperoxidase (MPO), zona occludens-1(ZO-1), and caspase-3 immunostaining revealed different degrees of preservation damage in all grafts.

**Conclusions:** Our novel assay system is simple and can test multiple solutions. It should therefore be a powerful tool for developing and improving new heart-graft preservation solutions.



Ann Sug. 2012. Jun11 [Epub ahead of print]

Transplantation of engineered chimeric liver with autologous hepatocytes and xenobiotic scaffold.

Hata T, Uemoto S, Fujimoto Y, Murakami T, Tateno C, Yoshizato K, Kobayashi E.

**Objective:**

Generation of human livers in pigs might improve the serious shortage of grafts for human liver transplantation, as well as enable liver transplantation without the need for deceased or living donors. We developed a chimeric liver (CL) by repopulation of rat hepatocytes in a mouse and successfully transplanted it into a rat recipient with vessel reconstruction. This study was designed to investigate the feasibility of CL for supporting the recipient after auxiliary liver grafting.

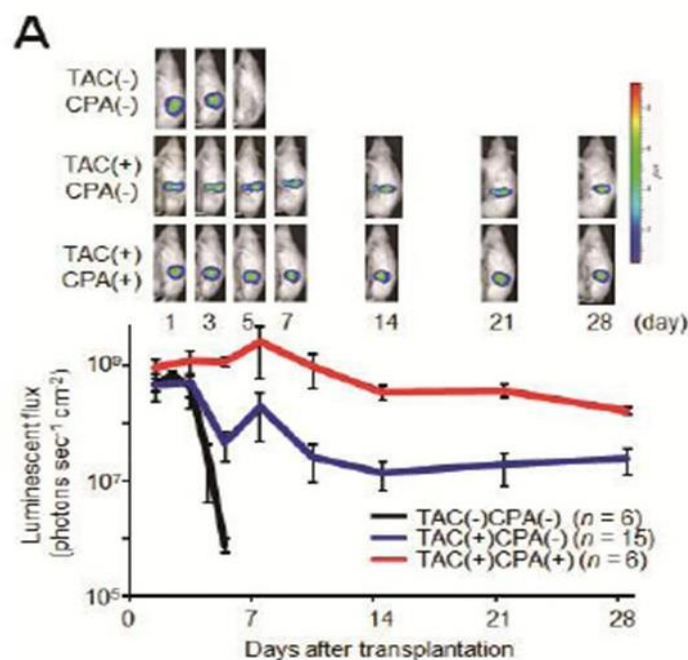
**Methods:**

Hepatocytes from luciferase transgenic or luciferase/LacZ double-transgenic rats were transplanted into 20-30-day-old urokinase-type plasminogen activator/severe-combined immunodeficiency (uPA/SCID) mice (n=40) to create CLs with rat-origin hepatocytes. After replacement of mouse hepatocytes with those from rats, the CLs were transplanted into wild-type Lewis (n=30) and analbuminemia (n=10) rats, followed by immunosuppression using tacrolimus with/without cyclophosphamide or no immunosuppression. Organ viability was traced by *in vivo* bioimaging and Doppler ultrasonography in the recipient rats for 4-6 months. Rat albumin production was also evaluated in the analbuminemia rats for 4 months. In addition, histological analyses including Ki67 proliferation staining were performed in some recipients.

**Results:**

Both immunosuppressive protocols significantly improved graft survival and histological rejection of CLs as compared to the non-immunosuppressed group. While rat albumin production was maintained in the recipients for 4 months after transplantation, ultrasonography revealed patent circulation in the grafts for 6 months. Ki67 staining analysis also revealed the regenerative potential of CLs after a hepatectomy of the host native liver, whereas immune reactions still remained in the mouse-origin structures.

**Conclusions:** This is the first report showing that engineered CLs have potential as alternative grafts to replace the use of grafts from human donors.



Stem cell. 2012 Apr 4. Doi:10.1002/stem.1161[Epub ahead of print]

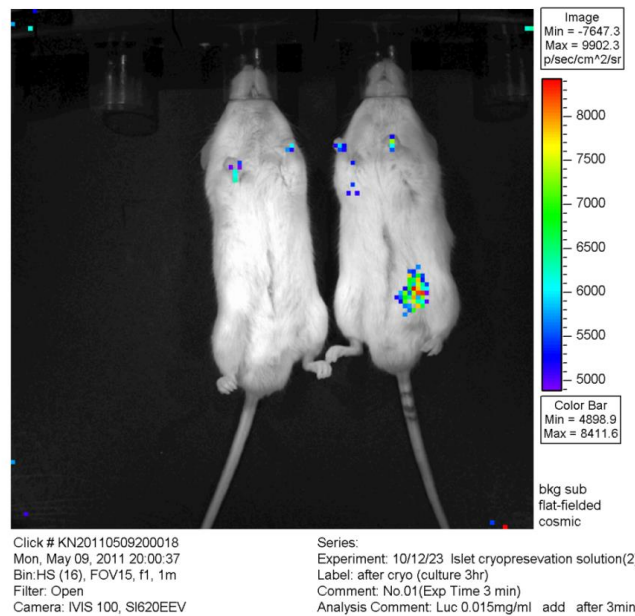
## Xenotransplanted embryonic kidney provides a niche for endogenous mesenchymal stem cell differentiation into erythropoietin-producing tissue.

Matsumoto K., Yokoo T., Matsunari H., Iwai S., Yokote S., **Teratani T.**, Gheisari Y., Tsuji O., Okano H., Utsunomiya Y., Hosoya T., Okano HJ., Nagashima H., **Kobayashi E.**:

### Abstract

Recent findings have demonstrated that stem cells can differentiate into mature tissue when supplied with a niche containing factors identical to those in the normal developmental program. A niche for the development of an organ can be provided by xenotransplantation of a similar developing organ. However, this process has many technical, safety and ethical concerns. Here, we established xenotransplantation models that control endogenous mesenchymal stem cell (MSC) differentiation into mature erythropoietin (EPO)-producing tissue in a niche provided by a developing xenometanephros. Transplantation of rat metanephroi into mouse omentum, and similarly pig metanephroi into cat omentum, led to the recruitment of host cells and EPO production. EPO-expressing cells were not differentiated from integrating vessels because they did not co-express endothelial markers (Tie-2 and VE-cadherin). Instead, EPO-expressing cells were shown to be derived from circulating host cells, as shown by EGFP expression in the grown transplants of chimeric mice bearing bone marrow from a transgenic mouse expressing

EGFP under the control of the EPO promoter. These results suggest that donor cell recruitment and differentiation in a xenotransplanted developing organ may be consistent between species. The cells responsible for EPO expression were identified as MSCs by injecting human bone marrow-derived MSCs and endothelial progenitor cells into NOD/SCID mice. Furthermore, using metanephroi from transgenic ER/E2F1 suicide-inducible mice, the xeno-tissue component could be eliminated, leaving autologous EPO-producing tissue. Our findings may alleviate adverse effects due to long-lasting immunosuppression and help mitigate ethical concerns.



*PLoS One*. 2012 Mar 21;7(3):e33157

## Impact of Normothermic Preservation with Extracellular Type Solution Containing Trehalose on Rat Kidney Grafting from a Cardiac Death Donor

Satomi Iwai, Takeshi Kikuchi, Naoya Kasahara, Takumi Teratani, Takashi Yokoo, Iwao Sakonju, Shouzou Okano, Eiji Kobayashi

### Background

The aim of this study was to investigate factors that may improve the condition of a marginal kidney preserved with a normothermic solution following cardiac death (CD) in a model of rat kidney transplantation (RTx).

## Methods

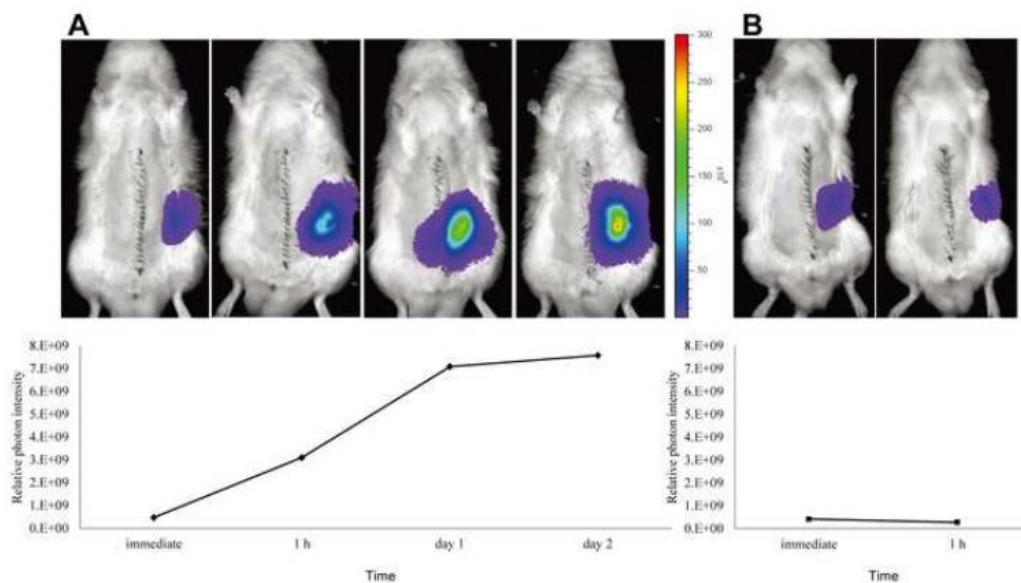
Post-euthanasia, Lewis (LEW) donor rats were left for 1 h in a 23°C room. These critical kidney grafts were preserved in University of Wisconsin (UW), lactate Ringer's (LR), or extracellular-trehalose-Kyoto (ETK) solution, followed by intracellular-trehalose-Kyoto (ITK) solution at 4, 23, or 37°C for another 1 h, and finally transplanted into bilaterally nephrectomized LEW recipient rats (n = 4–6). Grafts of rats surviving to day 14 after RTx were evaluated by histopathological examination. The energy activity of these marginal rat kidneys was measured by high-performance liquid chromatography (HPLC; n = 4 per group) and fluorescence intensity assay (n = 6 per group) after preservation with UW or ETK solutions at each temperature. Finally, the transplanted kidney was assessed by an *in vivo* luciferase imaging system (n = 2).

## Results

Using the 1-h normothermic preservation of post-CD kidneys, five out of six recipients in the ETK group survived until 14 days, in contrast to zero out of six in the UW group ( $p < 0.01$ ). Preservation with ITK rather than ETK at 23°C tended to have an inferior effect on recipient survival ( $p = 0.12$ ). Energy activities of the fresh donor kidneys decreased in a temperature-dependent manner, while those of post-CD kidneys remained at the lower level. ETK was superior to UW in protecting against edema of the post-CD kidneys at the higher temperature. Luminescence intensity of successful grafts recovered within 1 h, while the intensity of grafts of deceased recipients did not change at 1 h post-reperfusion.

## Conclusions

Normothermic storage with extracellular-type solution containing trehalose might prevent reperfusion injury due to temperature-dependent tissue edema



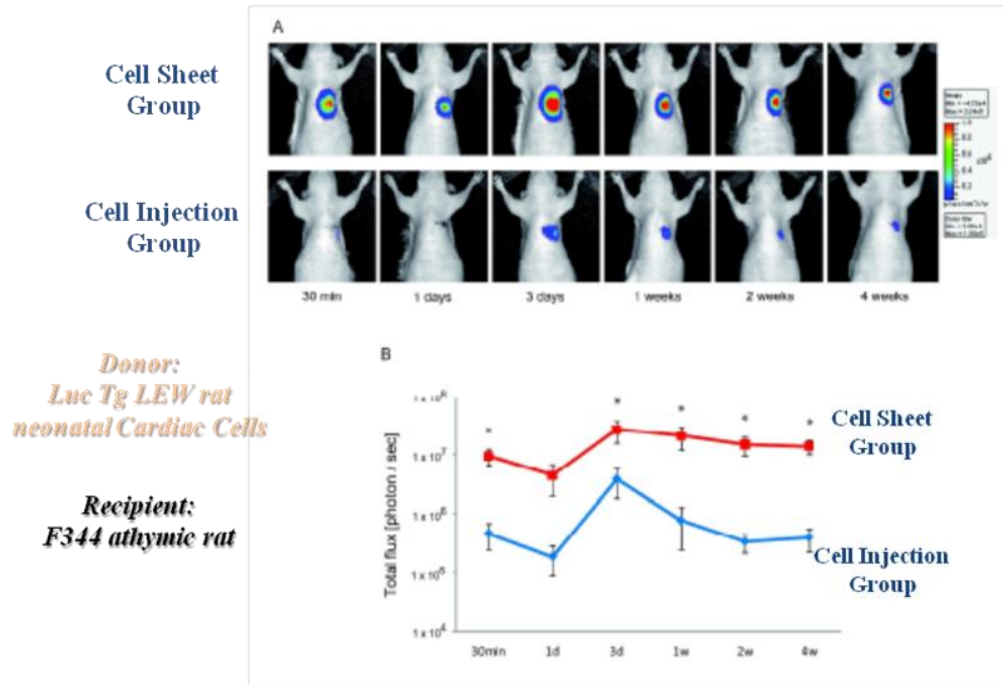
Tissue Eng Part A. 2011 Dec;17(23-24):2973-80. Epub 2011 Aug 29.

## **Cardiac cell sheet transplantation improves damaged heart function via superior cell survival in comparison with dissociated cell injection.**

Sekine H, Shimizu T, Dobashi I, Matsuura K, Hagiwara N, Takahashi M, **Kobayashi E**, Yamato M, Okano T.

### **Abstract**

Regenerative therapies have currently emerged as one of the most promising treatments for repair of the damaged heart. Recently, numerous researchers reported that isolated cell injection treatments can improve heart function in myocardial infarction models. However, significant cell loss due to primary hypoxia or cell wash-out and difficulty to control the location of the grafted cells remains problem. As an attempt to overcome these limitations, we have proposed cell sheet-based tissue engineering, which involves stacking confluent cultured cells (two-dimensional), cell sheets, to construct three-dimensional cell-dense tissues. Cell sheet transplantation has been able to recover damaged heart function. However, no detailed analysis for transplanted cell survival has been previously performed. The present study compared the survival of cardiac cell sheet transplantation to direct cell injection in a rat myocardial infarction model. Luciferase-expressing neonatal rat cardiac cells were harvested as cell sheets from temperature-responsive culture dishes. The transplantation of cell sheets was compared to the direct injection of isolated cells dissociated with trypsin-ethylenediaminetetraacetic acid. These grafts were transplanted to infarcted rat hearts and cardiac function was assessed by echocardiography at 2 and 4 weeks after transplantation. In vivo bioluminescence and histological analyses were performed to examine cell survival. Cell sheet transplantation consistently yielded greater cell survival than cell injection. Immunohistochemistry revealed that cardiac cell sheets existed over the infarcted area as an intact layer. In contrast, the injected cells were scattered with relatively few cardiomyocytes in the infarcted areas. Four weeks after transplantation, cardiac function was also significantly improved in the cell sheet transplantation group compared with the cell injection. Twenty-four hours after cell grafting, significantly greater numbers of mature capillaries were also observed in the cardiac cell sheet transplantation. Additionally, the numbers of apoptotic cells with deterioration of integrin-mediated attachment were significantly lower after cardiac cell sheet transplantation. In accordance with increased cell survival, cardiac function was significantly improved after cardiac cell sheet transplantation in comparison to cell injection. Cell sheet transplantation can repair damaged hearts through improved cell survival and should become a promising therapy in cardiovascular regenerative medicine



PLoS One. 2011 Apr 29;6(4):e19195.

## Bone marrow-derived mesenchymal stem cells ameliorate hepatic ischemia reperfusion injury in a rat model.

Kanazawa H, Fujimoto Y, Teratani T, Iwasaki J, Kasahara N, Negishi K, Tsuruyama T, Uemoto S, Kobayashi E.

### Abstract

#### BACKGROUND:

Ischemia-reperfusion (I/R) injury associated with living donor liver transplantation impairs liver graft regeneration. Mesenchymal stem cells (MSCs) are potential cell therapeutic targets for liver disease. In this study, we demonstrate the impact of MSCs against hepatic I/R injury and hepatectomy.

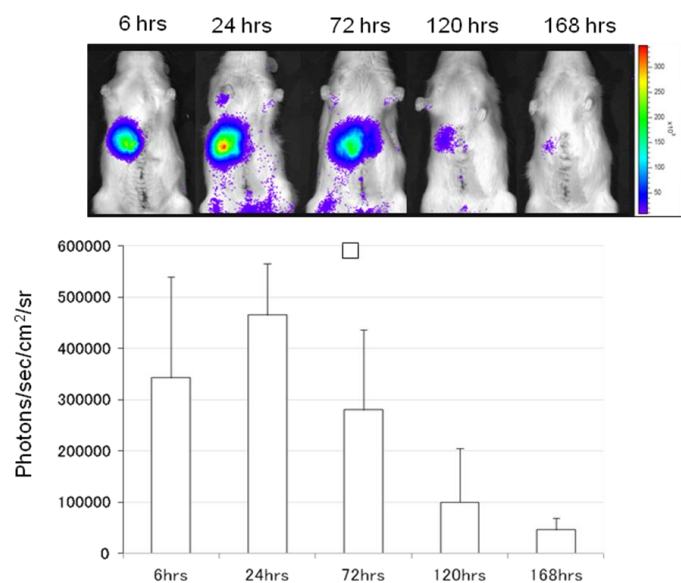
#### METHODOLOGY/PRINCIPAL FINDINGS:

We used a new rat model in which major hepatectomy with I/R injury was performed. Male Lewis rats were separated into two groups: an MSC group given MSCs after reperfusion as treatment, and a Control group given phosphate-buffered saline after reperfusion as placebo. The results of liver function tests, pathologic changes in the liver, and the remnant liver regeneration rate were assessed. The fate of transplanted MSCs in the luciferase-expressing rats was examined by in vivo luminescent imaging. The MSC group showed peak luciferase activity of transplanted MSCs in the remnant liver 24 h after

reperfusion, after which luciferase activity gradually declined. The elevation of serum alanine transaminase levels was significantly reduced by MSC injection. Histopathological findings showed that vacuolar change was lower in the MSC group compared to the Control group. In addition, a significantly lower percentage of TUNEL-positive cells was observed in the MSC group compared with the controls. Remnant liver regeneration rate was accelerated in the MSC group.

#### CONCLUSIONS/SIGNIFICANCE:

These data suggest that MSC transplantation provides trophic support to the I/R-injured liver by inhibiting hepatocellular apoptosis and by stimulating regeneration



*Islets*. 2011 May-Jun;3(3):111-7. Epub 2011 May 1.

### **Luminescence technology in preservation and transplantation for rat islet.**

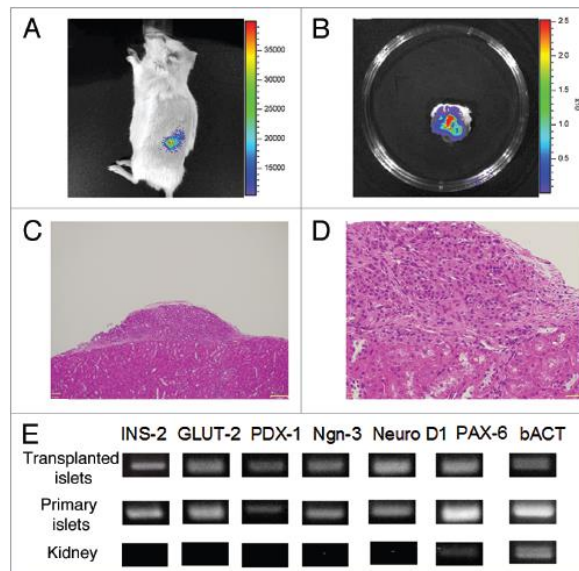
Negishi K, **Teratani T**, Iwasaki J, Kanazawa H, Kasahara N, Lefor AT, Uemoto S, **Fujimoto Y**, **Kobayashi E**.

#### **Abstract**

The development of organ preservation solutions and associated technology has been a major effort in tissue transplantation recently. However, this research takes a great deal of time and resources. In this study, a novel method for the evaluation of preservation solutions was established by using islet cells. Primary islets were obtained by hand-picking method from the luciferase transgenic (Luc-Tg) rat



pancreas. The viability rate and living condition of islets preserved with several solutions were evaluated by relative photon intensity. Preserved islets were transplanted to the renal capsule of streptozotocin (STZ)-induced type 1 diabetic NOD-scid mouse, and the intraperitoneal glucose tolerance test (IPGTT) and histology were analyzed. The Luc-Tg rat islet viability was increased in a relative photon intensity-dependent manner. In the recipients of ET-Kyoto (ET-K) or University of Wisconsin (UW) solution preserved Luc-Tg rat islet at 1 day, hyperglycemia induced by glucose injection declined to the normal range. In conclusion, this study demonstrates that the ET-K preservation method allowed tissue ATP synthesis and amelioration of cold ischemic tissues damage during extended 24 h isolated-islet preservation. This simple method will be adapted easily to the clinical setting and used to maximize the utilization of islet transplantation as well as for pancreas sharing with remote centers.



Plast Reconstr Surg. 2011 Jan;127(1):78-87.

## **Luminescence imaging of regenerating free bone graft in rats.**

Yamaguchi A, Murakami T, Takahashi M, **Kobayashi E**, Sugawara Y.

### **Abstract**

#### **BACKGROUND:**

Bone transplantation is an important procedure often used for bone defect reconstruction after trauma and malignancies. However, the kinetics of free bone graft-derived cells remains unclarified. The authors examined the kinetics of graft-derived cells using transgenic rats systemically expressing firefly luciferase.

## METHODS:

Free iliac bone grafts ( $5 \times 5 \times 2$  mm,  $n = 10$ ) derived from luciferase transgenic rats were transplanted into the subcutaneous space of the back of wild-type Lewis rats, and the kinetics of graft-derived cells were evaluated over time by determining the level of luminescence emission.

## RESULTS:

Although the luminescence level emitted by luciferase decreased after transplantation, a substantial luminescence level (mean,  $1.6 \times 10^7$  photons/second) was emitted from donor-derived cells even at 180 days after transplantation, suggesting a long-term survival of graft-derived cells. In a computed tomographic image analysis of bone grafts retrieved 180 days after transplantation, high-luminescence grafts with a sufficient number of viable graft-derived cells (mean,  $2.6 \times 10^7$  photons/second;  $n = 4$ ) showed significantly higher bone graft volume (3.1-fold) and polar moment of inertia of area (7.2-fold) than low-luminescence grafts (mean,  $1.0 \times 10^7$  photons/second;  $n = 4$ ), indicating that high-luminescence grafts maintain better conditions.

## CONCLUSION:

These results suggest that bone graft-derived cells can survive for a long time and that the presence of a sufficient number of viable graft-derived cells is essential for graft engraftment and remodeling

