



# Patella cryo-free technique with recycled frozen autograft reconstruction preserves extensor mechanism for proximal tibial malignancy

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## Abstract

**Backgrounds:** We designed a patella cryo-free method to protect patella from cryoinjury during recycled frozen bone-prosthesis-composite reconstruction for proximal tibial malignancy. This study aimed to use animal model to ensure safety and efficacy of this method and reported our clinical outcomes.

**Methods:** Six swine proximal tibias along with patella and patellar tendon were harvested and divided into group A (n = 3, traditional patella freezing) and group B (n = 3, patella cryo-free). Temperature curve measurement, histological analysis, and TUNEL assay were performed in both groups. Clinically, we retrospectively reviewed 23 patients with proximal tibia malignant bone tumor (13: traditional patella freezing method; 10: patella cryo-free method). The clinical and functional outcomes were reported and compared in both groups.

**Results:** Temperature curve of the group B showed that ideal therapeutic temperature (<-60°C) required to kill tumor cells can be achieved in the proximal tibia while the innocent patella can be kept in room temperature at all time. Histological analysis showed better preservation of the cartilage tissue in patella of group B. TUNEL assay showed significantly more apoptotic cells in the frozen tibia of both groups and frozen patella of group A. When reviewing our clinical results, less complication of the patella as well as better functional preservation were found in patients subjecting to patella cryo-free method. No local recurrence was observed in either group.

**Conclusion:** Patellar cryo-free technique could protect patella from cryoinjury during freezing and therefore preserve more extensor functions for patients with proximal tibial malignant bone tumors.

**Keywords:** Biological reconstruction; Limb salvage; Liquid nitrogen; Proximal tibial bone tumor; Recycled bone

## 1. INTRODUCTION

The proximal tibia is the second most common site of primary bone sarcomas. In our institute, we prefer recycled bone-prosthesis-composite (BPC) is preferred in our institute if bony integrity is intact. Because it could restore bone stock, avoid complications of osteoarticular bone graft, and preserve normal function of the knee joint.<sup>1-4</sup>

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We routinely perform patellar chevron osteotomy during tumor excision rather than detaching the patellar tendon to maintain more extensor function. The resected patella and its tendon and proximal tibia were subjected to irradiation or freezing and arthroplasty reconstruction. After seating the BPC to the host bone, we would fix the patella with screws and wires. In our previous experiences, complications of the patella occurred during the follow-up, including fixation failure, malunion or nonunion of the patella, and early cartilage degeneration, which was probably resulted from a lack of effective methods to protect the innocent patella. And bone and cartilage tissue would be injured by either irradiation<sup>5-7</sup> or LN freezing,<sup>7-9</sup> which may negatively affect healing of the patella and damage the patellar articular cartilage in the long term. Therefore, we designed a new cryo-free method with a freezing tank intended to protect the resected patella from cryo-injury during LN freezing.

The purpose of this study aimed to ensure the safety and histological efficacy of the patella cryo-free and traditional patella freezing methods on the porcine model. Clinically, we would like to retrospectively review our clinical experiences of these two methods and report clinical and functional outcomes.

## 2. METHODS

### 2.1. Study design

The study is composed of two parts. The first part is to acquire the temperature curve and histological validation to compare the safety and efficacy of traditional patella freezing and patella cryo-free methods on the porcine model. The second part is to review the clinical results of both approaches. We did not finish our first part of the study on a human model because inserting probes of a digital thermometer or harvesting specimens on patients' proximal tibia and patella bone may increase the risks of tumor contamination and unnecessary bone loss. Therefore, we used the porcine model for temperature measurement and histological validation of the patella and its cartilage tissue. We have included ARRIVE and STROBE checklists to show that we have conformed to the ARRIVE and STROBE guidelines.

### 2.2. Surgical treatment for recycled autograft reconstruction for proximal tibia malignant bone tumors

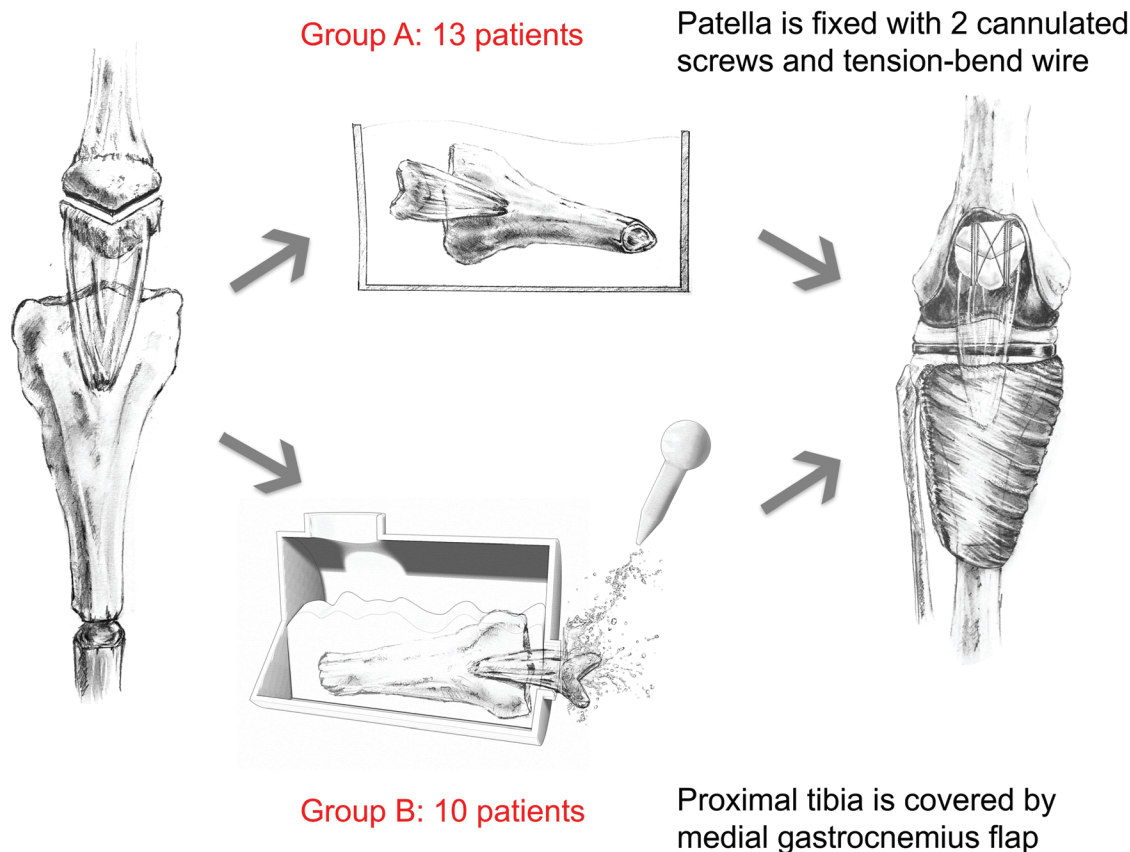
Patellar V-shaped chevron osteotomy with a 120° angle was performed at the lower pole of the patella in all cases during tumor resection.<sup>4</sup> After tumor resection, the tumor-bearing proximal tibia with the resected patella and patellar tendon were all immersed in LN for 20 minutes in group A. In group B, the proximal tibia was then put into a freezing tank (ISTAR VISION CO., LTD., Taiwan) that was specially designed for LN freezing during recycled autograft reconstruction and approved by TFDA (MOHW-MD-(I)-No, 008254). The innocent patella and

patellar tendon were held outside the side aperture of the tank, and the aperture was further sealed with a silicone band and sterilized petroleum jelly to prevent leakage of LN during freezing. The patella and patellar tendon, kept outside the freezing tank, were rinsed with warm sterilized water during freezing. After freezing, the bones in both groups were thawed slowly at room temperature for another 20 minutes and then rinsed with sterilized water to room temperature.

BPC preparation with NexGen RHK Knee prosthesis (Zimmer, Warsaw, IN, USA) was applied in all patients as previous report.<sup>4</sup> After the final seating of the prosthesis composite on the tibia, the patella was fixed with cannulated screws and tension-band wiring. Medial gastrocnemius muscle flap transfer was used routinely in all cases. The reconstruction of BPC, fixation of the resected patella, and preparation of the medial gastrocnemius muscle flap are shown in Fig. 1.

### 2.3. Porcine bone preparation

We obtained porcine proximal tibias along with patella and patellar tendon from a legal slaughterhouse for meat processing. In brief, after preparing well, a legal meat manufacturer would subject swine (8 months of age) to electronacrosis by applying 200 V of high-frequency alternating current (about 1500 Hz) to the head to produce electric shock. The animal was then unconscious, and six proximal tibias along with the resected-patella and patellar tendon were obtained from six swine during meat processing and divided into two groups randomly (group A, n = 3, traditional patella freezing method; group B, n = 3, patella



**Fig. 1** Illustration of surgical procedures in both groups. Patella chevron osteotomy was performed during wide excision of the proximal tibia bone tumor. During cryotherapy, the proximal tibia along with the innocent patella and patella tendon was immersed in LN during freezing (group A). In group B, the innocent patella was kept outside the freezing tank and rinsed with sterilized warm water during freezing. After the final seating of the prosthesis composite on the tibia, the patella was fixed with cannulated screws and tension-band wiring. Medial gastrocnemius muscle flap transfer was used routinely in all cases.

cryo-free method). Bone and cartilage tissue were obtained before cryotherapy as a control group. LN freezing was then performed immediately in a separate lab in the slaughterhouse.

#### 2.4. Cryotherapy and temperature curve measurement of patella cryo-free method

Small holes were made on the center of the proximal tibia, its tuberosity, and patella of group B by 2.5 mm drill to insert detectors of digital thermometer. We recorded the temperature every 30 seconds to obtain temperature curves of the patella, tibia tuberosity, and proximal tibia bone during cryotherapy (n = 3). In group A, we did not measure the temperature curve because the tibia and patella were both immersed into LN immediately during freezing.

#### 2.5. Histological analysis of patella cartilage degeneration

Specimens from the bone tissue of the proximal tibia, patella, and patellar cartilage of both groups were harvested before cryotherapy as a control group and after cryotherapy as an experimental group (n = 6, two samples × three porcine bones). Two orthopedic pathologists of our hospital were invited to examine each sample, and they were blinded to groups A and B. We evaluated the viability and degeneration of articular chondrocytes of the resected patella of both methods. Tissue slides and the stain were performed as mentioned in the works of literature.<sup>10,11</sup> The degeneration of the articular chondrocytes after cryotherapy was defined as vacuolation or lacuna formation of cytoplasm with shrinkage or absence of the nucleus.<sup>10-12</sup> The example of calculating degenerated chondrocytes compared to all was illustrated in Fig. 2.

#### 2.6. TUNEL assay

During cryotherapy, either normal tissue or tumor cells would undergo both apoptotic pathways.<sup>13,14</sup> Therefore, we performed a TUNEL assay on bone tissue and articular cartilage of tibia and patella in both groups for apoptosis analysis (n = 6, two samples × 3 porcine bone or cartilage tissue). APO-BrdU TUNEL Assay Kit (Thermo Fisher Scientific, Waltham, MA) was applied according to the manufacturer's instructions to mark apoptotic DNA fragments with green fluorescence.<sup>15</sup> The slides were then stained by Vectashield Mounting Medium with DAPI (4',6-diamidino-2-phenylindole; Vector Laboratories, Burlingame, CA) to mark the nucleus of both live and apoptotic cells with blue fluorescence.<sup>16</sup> Quantification of TUNEL-positive

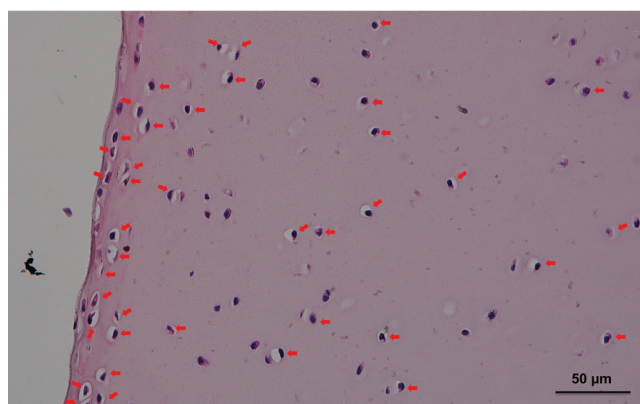
apoptotic cells (green color) and all nuclear cells (blue color) were obtained with Image J version 1.5 (National Institutes of Health) software. The percentage of TUNEL-positive apoptotic cells among all nuclear cells was analyzed and compared between groups.

#### 2.7. Patients selection

From January 2008 to December 2017, we retrospectively reviewed 23 patients of proximal tibial malignancy in our institute (20 osteosarcomas, one parosteal osteosarcoma, one undifferentiated high-grade sarcoma, and one malignant giant cell tumor). All of them had no pathological fracture, no patella, and its tendon involvement preoperatively. They all received tumor excision and frozen recycled BPC reconstruction (13: traditional patella freezing, group A; 10: patella cryo-free method, group B). Before 2010, the first three patients all received conventional patella freezing during cryotherapy because the patella cryo-free technique had not yet been developed. After 2010, we started to use freezing tank (proved by TFDA) (MOHW-MD-(I)-No, 008254), and the patella cryo-free technique was applied more frequently. Since then, we had a randomized selection between the two methods, and the demographic characteristics found no differences among the analyzed criteria in the studied populations (Table 1). A minimum follow-up period of 3 years was required. No patients in either group had pre-existing osteoarthritis over the patella-femoral joint. The study project was approved by the Institutional Review Board of Taipei Veterans General Hospital (IRB-TPEVGH No.:2016-05-013CC).

#### 2.8. Rehabilitation and clinical evaluation

All patients commenced with range-of-motion (ROM) training right after the surgery and partial weight-bearing 3 months postoperatively. Quadriceps exercises were started 6 weeks postoperatively. All patients then received follow-up every 3 months for the next 5 years and annually thereafter. Functional evaluation (ROM and Musculoskeletal Tumor Society [MSTS] scores) and image studies (plain film, chest computed tomography and magnetic resonance imaging) were performed every visit. The patellar complication was defined as patellar malunion or nonunion and bone resorption of the lower pole of the patella.



**Fig. 2** Measurement of nucleus degeneration and vacuolation of articular chondrocytes at the cartilage layer. The total number of nucleated chondrocytes is 80. The total number of degenerated and vacuolated chondrocytes is 38 (arrow). The percentage of degenerated chondrocytes was  $38/80 \times 100\% = 47.5\%$ .

**Table 1**

**Patient profiles and tumor characteristics**

	Group A (patella freezing, n = 13)	Group B (patella cryo-free, n = 10)	p
Sex			0.645 <sup>a</sup>
Male	9	6	
Female	4	4	
Age			0.322 <sup>b</sup>
Years (mean ± SD)	27.3 ± 13.11	22.1 ± 10.4	
Q1-Q3	20.0-29.0	16.3-23.3	
Diagnosis			
Osteosarcoma	11	8	
Parosteal osteosarcoma	1	1	
Malignant giant cell tumor	0	1	
Undifferentiated high-grade sarcoma	1	0	
Staging AJCC			0.840 <sup>a</sup>
IB	1	1	
IIA	4	2	
IIB	8	7	

AJCC = American Joint Committee on Cancer.

<sup>a</sup> $\chi^2$  test.

<sup>b</sup>Mann-Whitney U test.



## 2.9. Statistical analysis

All statistical analyses for histological data were done with GraphPad Prism software (GraphPad Software, San Diego, CA). For parametric data, we used one-way analysis of variance (ANOVA) test with post hoc Bonferroni's test for analysis. For non-parametric data, we used Kruskal-Wallis method and Dunn's post comparison test for analysis. A *p*-value of the one-way ANOVA test or Kruskal-Wallis test was considered as statistically significant when  $<0.05$ , very significant when  $<0.01$ , and extremely significant when  $<0.001$ . For clinical data analysis, demographic variables were examined using  $\chi^2$  test (sex and tumor staging) and Mann-Whitney U test (age and follow-up time), respectively. The complications in both groups were analyzed using  $\chi^2$  test. The postoperative knee joint ROM, MSTs scores, and local recurrence rates were compared using the Mann-Whitney U test. All tests were two-sided, and a *p*-value  $<0.05$  was considered statistically significant.

## 3. RESULTS

### 3.1. Temperature curve of patella cryo-free method

Temperature curves of group B were obtained and shown in Fig. 3. The proximal tibia bone and its tuberosity can reach  $-60^\circ\text{C}$  in about 90 and 240 seconds with a mean rate of  $-57.9^\circ\text{C}$  and  $-21.0^\circ\text{C}$  per minute, respectively, and then decline shortly to temperatures as low as  $-196^\circ\text{C}$ . When being rinsed with sterilized warm water, the temperature of the innocent patella can be maintained at room temperature ( $24\text{--}25^\circ\text{C}$ ) throughout the whole course.

### 3.2. Histological analysis of porcine patellar cartilage tissue

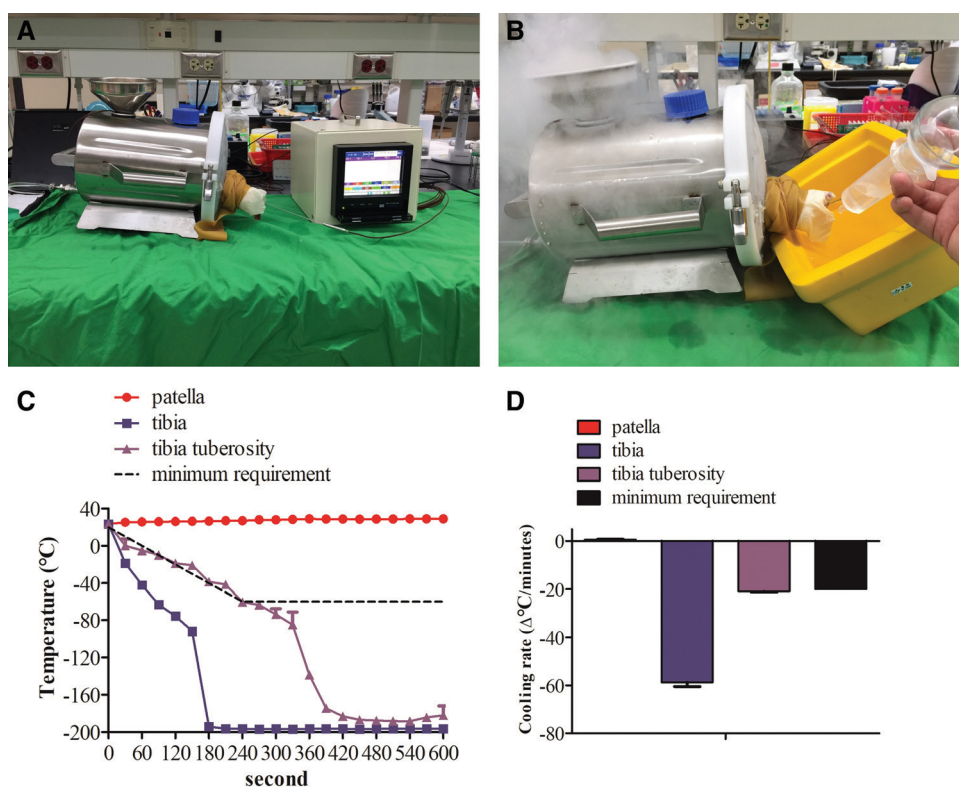
Histological evaluation of the chondrocytes of the articular cartilage from the innocent patella was performed under  $20\times$  microscopic examination. The percentage of degenerated chondrocytes of articular cartilage was significantly higher in the frozen patella of group A ( $42.9\pm 3.9\%$ , Fig. 4B) than the control group ( $8.6\pm 1.0\%$ , Fig. 4A) and cryo-free patella of group B ( $13.2\pm 3.9\%$ , Fig. 4C). The *p*-value was 0.0013 after analysis with the Kruskal-Wallis test (non-parametric), and the post hoc analysis also showed extremely significant ( $p < 0.001$ ) between frozen and non-frozen patella cartilage (Fig. 4D).

### 3.3. TUNEL assay

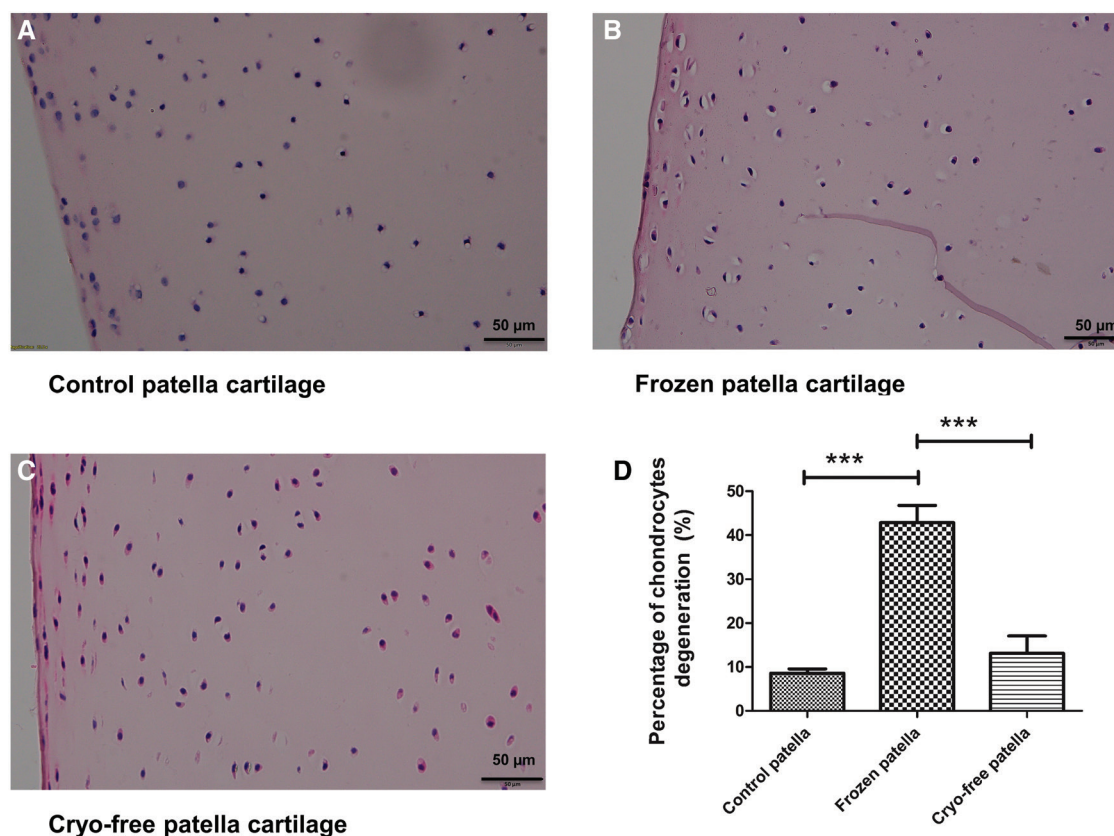
In the control group, only a few TUNEL-positive apoptotic cells were observed in the tibia ( $2.7\pm 0.5\%$ ), patella ( $1.9\pm 0.6\%$ ), and patellar cartilage ( $1.2\pm 0.9\%$ ) (Fig. 5A). In Group A, the TUNEL-positive cells were higher in the tibia ( $97.5\pm 1.6\%$ ), patella ( $96.3\pm 2.1\%$ ), and patellar cartilage ( $97.1\pm 2.0\%$ ) (Fig. 5B). In group B, the TUNEL-positive cells were higher in the tibia ( $95.6\pm 1.1\%$ ), but significantly fewer in the patella ( $4.0\pm 1.6\%$ ) and its cartilage ( $1.6\pm 1.3\%$ ) (Fig. 5C). The *p*-value was  $<0.001$  after analysis with one-way ANOVA test (parametric), and the post hoc analysis also showed extreme significance ( $p < 0.001$ ) between frozen and non-frozen tissue (Fig. 5D).

### 3.4. Clinical results

After a mean follow-up time of 79.2 months in group A and 77.1 months in group B, three patients in group A and two patients in group B developed lung metastasis, and four of them died. The other 17 patients survived and were disease-free. No



**Fig. 3** A-D, Measurement of temperature curve and cooling rate of the patella cryo-free method. A, Illustration of the setting of the digital thermometer during the patella cryo-free method. Three sensors were inserted into the innocent patella, tibia tuberosity, and proximal tibia. B, During the LN freezing, the innocent patella was rinsed with warm water throughout the whole course. C, The temperature curve showed that the innocent patella could be kept at room temperature, while the proximal tibia and tibia tuberosity both met the temperature required to kill tumor cells. D, The cooling rate of the different parts of the bone. The proximal tibia and tibia tuberosity both met the temperature required to kill tumor cells.



**Fig. 4** A-D, Histological analysis of porcine patella cartilage tissue. A, Control group shows only very few degenerated chondrocytes. B, After cryotherapy, the frozen patella of group A shows significant numbers of degenerated chondrocytes, which is characterized by nucleus shrinkage with concomitant vacuolation of the cytoplasm. Some empty lacunas can also be observed within the cartilage matrix. C, In contrast, the cryo-free patella of group B shows limited numbers of degenerated chondrocytes. D, A bar chart shows the measurement of degenerated chondrocytes within the cartilage matrix. The microscopic magnification was  $\times 40$ . Dunn's post comparison test was performed.  $*p < 0.05$ ;  $**p < 0.01$ ;  $***p < 0.001$ .

local recurrence was observed among any patients in either group (Table 2).

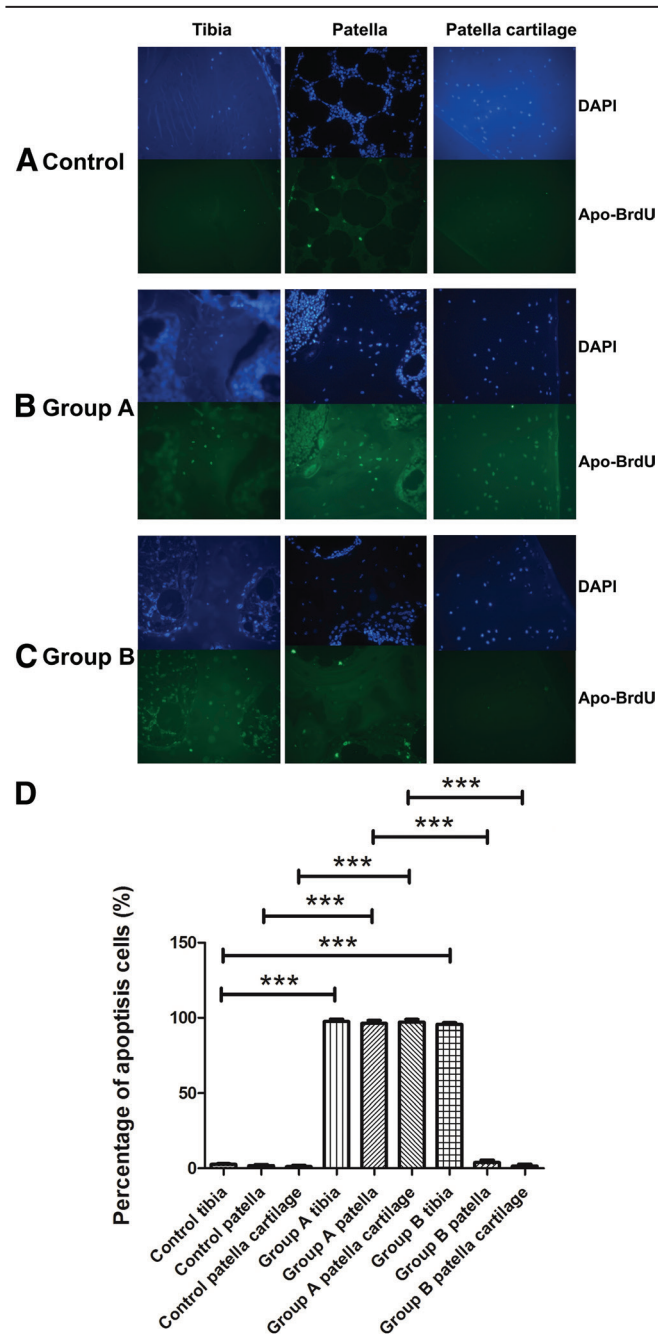
Clinical and functional results are shown in Table 2. The latest imaging study revealed fewer patella-related complications in group B than in group A. Eight patients in group A had malunion/nonunion (5) or bone resorption (3) of the resected patella while only one in group B had patellar malunion ( $p = 0.012$ ). The mean extension lag degrees were  $21.2^\circ$  in group A and  $6.0^\circ$  in group B ( $p < 0.001$ ). The mean flexion degrees were  $98.7^\circ$  in group A and  $113.5^\circ$  in group B ( $p < 0.001$ ). At the last follow-up, the mean MSTS score was higher in group B (mean, 93.33%) than in group A (mean, 82.56%) with  $p = 0.001$ . Case examples from each group are shown in Figs. 6 and 7. Of the 23 patients, only four had superficial wound infection (three in group A and 1 in group B,  $p = 0.412$ ), but all healed well after surgical debridement and local flap treatment. No neurovascular injury, associated fracture, avulsion of patella tendon at tibial tuberosity, or tibial nonunion occurred in either group.

#### 4. DISCUSSION

Reconstruction of the extension mechanism of the knee is challenging during the limb salvage procedure for proximal tibial malignancy. When performing endoprosthesis reconstruction, the patellar tendon is detached from the proximal tibia during tumor excision, which increases the risk of tumor contamination if the tumor is very close to the tibial tuberosity. Furthermore,

during reconstruction, the detached patella is advanced to the prosthesis and reattached over the metal implant with Dacron tape or non-absorbable sutures.<sup>17-19</sup> Lack of solid and sustainable attachment of the patellar tendon on the metal implant would disrupt the extensor mechanism, and subsequent extension lags up to  $10^\circ$ , and  $30^\circ$  had been reported.<sup>20,21</sup> Therefore, we designed a V-shaped chevron osteotomy with a  $120^\circ$  angle during tumor excision of recycled autograft reconstruction. And the extensor mechanism can be theoretically restored after bone-to-bone healing of the patella. However, in our previous experiences, the complication of either irradiated or frozen patella occurred during the follow-up, including fixation failure, malunion or nonunion of the patella, and early cartilage degeneration, therefore we designed a patella cryo-free method to preserve the innocent patella and more extensor function.

During tumor eradicating procedure by freezing, the ideal temperature required to kill tumor cells during freezing is to reach a tissue temperature of  $-40^\circ\text{C}$  to  $-50^\circ\text{C}$  in the tumor, including a safe distance around it.<sup>13,14,22</sup> The cooling rate should be at least  $-20^\circ\text{C}$  per minute or more to enhance more formation of the intracellular ice and kill tumor cells.<sup>13,22</sup> When freezing in the tank, the proximal tibia achieved the temperature required to kill tumor cells with a freezing rate of  $-57.9^\circ\text{C}$  per minute. The adjacent structure, such as tibial tuberosity, also has a cool rate of  $-21.0^\circ\text{C}$  per minute in the first 4 minutes, and the temperature dropped even faster after that. Meanwhile, the innocent patella can be kept at room temperature throughout the freezing course when rinsing with sterilized warm water.



**Fig. 5** A-D, Application of TUNEL assay of porcine bone and cartilage tissue to confirm the histological analysis of both methods. DAPI stain helps to identify the nuclei of both live and dead cells, whereas Apo-BrdU stain only labels cells having DNA damage in the course of apoptosis. A, TUNEL assay of the control group shows negative results in Apo-BrdU stain of bone tissue of tibia and patella as well as articular cartilage of the patella. B, In group A, cellular apoptosis is observed in tibia, patella and articular surface of the patella cartilage. C, In group B, the application of patella cryo-free method successfully preserves the innocent tibia bone and cartilage tissue as demonstrated by negative Apo-BrdU stain. In contrast, the positive Apo-BrdU stain in proximal tibia also demonstrates efficacy of the method for freezing bone tissue and inducing further apoptosis. D, A bar chart shows the percentage of TUNEL-positive cells in proximal tibia, patella, and patella cartilage in 3 groups. Post hoc Bonferroni's test was performed. \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ .

This experiment proves that the patella cryo-free method can achieve selective freezing and preserve normal tissue from cryoinjury.

Song et al showed that ice formation was found into the lacunae of the cartilage matrix after freezing. Chondrocytes within the ice-filled lacunae appeared totally disrupted, with vacuolated cytoplasm and irregular nuclei.<sup>11</sup> Electron micrograph examination also had a similar finding.<sup>10</sup> These damaged and degenerated chondrocytes would sustain. They cannot be recovered or regenerated, as shown in a short-term report from Tanzawa et al (mean 19.1 months) and long-term reports from Hayashi et al (mean 94.0 months).<sup>8,23</sup> In the present study, we found freezing would damage the cartilage tissue of the patella of group A. Chondrocytes injury and degeneration were more prominent, and the results can also be confirmed by TUNEL assay.

During cryotherapy, intracellular ice crystals would damage the lipid layer of the cell and nucleus membrane and initiate apoptotic changes in regular tissue and tumor cells.<sup>13,22,24</sup> Therefore, we used the TUNEL assay to detect apoptosis in the tibia and patella after cryotherapy. We observed consistent apoptosis of porcine proximal tibia bone tissue in both groups, indicating that these two methods could efficiently damage normal bone cells as to malignant tumor cells in the tumor setting. In group B, many live cells were observed in the cryo-free patella, indicating that normal bone and cartilage tissue of the patella can be well preserved, and therefore reduced cryoinjury of the patella during freezing.

Clinically, the present study found no differences in local recurrence during the follow-up of both groups. The proximal tibias of both methods achieved temperatures as low as  $-196^{\circ}\text{C}$  in a very short time, which is far lower than the temperature required ( $<-60^{\circ}\text{C}$ ) to kill the tumor.<sup>25,26</sup> Our animal model and clinical experience demonstrated that carefully soaking all tumor components of both groups in LN achieved the ideal temperatures required to kill tumor cells. No differences were noted in the ability of tumor ablation in either the patella cryo-free or the patella freezing group.

The present study also demonstrated that the patella cryo-free method led to fewer patella-related complications and better clinical outcomes. Because we could efficiently preserve more live bone and cartilage tissue, the cryo-free patella healed better with significantly fewer complications, such as malunion and hardware migration. As such, patients of group B achieved better ROM of the knee joint, less extension lag, and significantly higher MSTS scores.

In our clinical series, the rate of other complications was similar in both groups, and no statistically significant differences were found. Because we did not detach patella tendon from the proximal tibia, there was no patella tendon avulsion in both groups. Although some patients in both groups had superficial infections, they all recovered nicely at the end of the follow-up. Routine use of rotational medial gastrocnemius muscle flap transfer after reconstruction also provided better soft tissue coverage over the frozen bone and helped to prevent future infection.<sup>27-29</sup>

The present study has several limitations. First, we did not build a porcine animal model of proximal tibial sarcoma. Only normal proximal tibia and patella tissue were used to simulate the surgical procedure we performed in the clinical setting. Second, patient data in our clinical study were analyzed retrospectively, and we could not rule out selection bias. Third, the limited number of patients may obscure the clinical results. Additional prospective, long-term studies are needed to evaluate further the safety and efficacy of the patella cryo-free method.

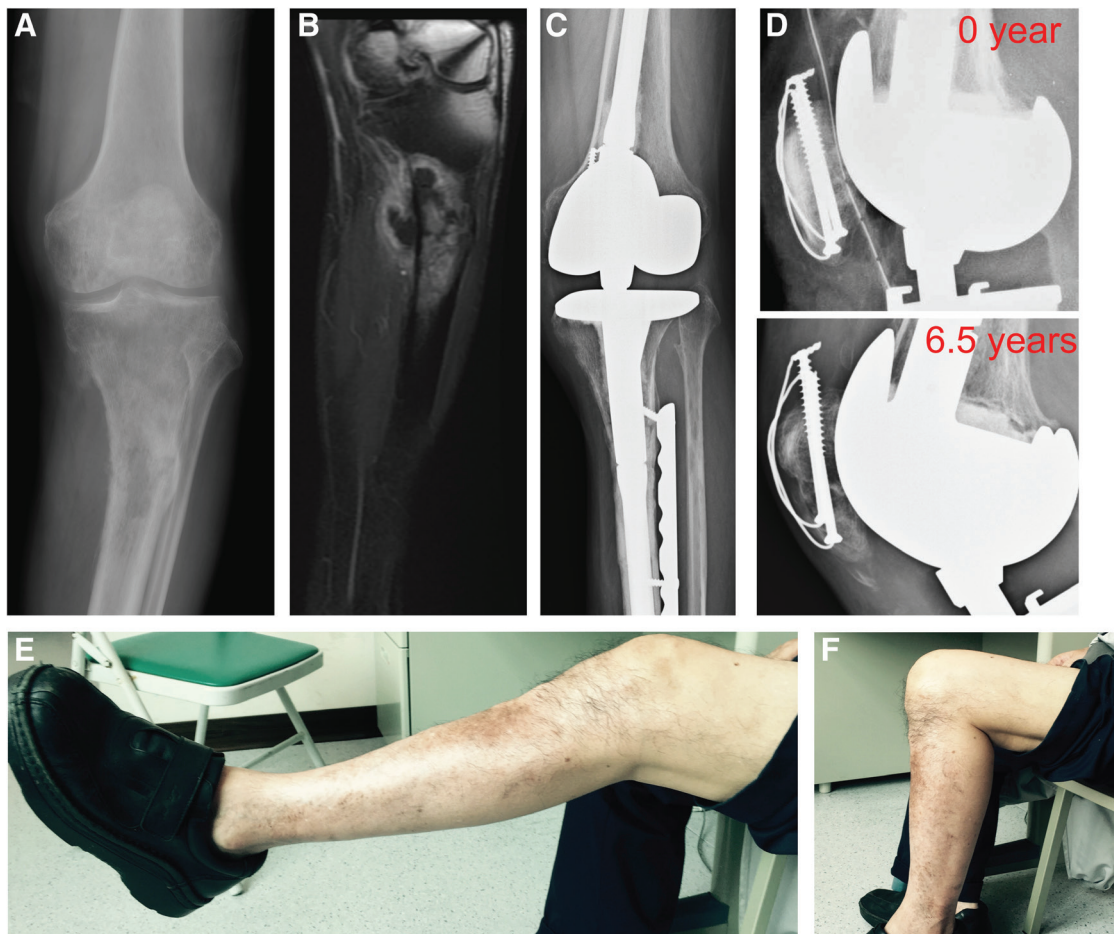
In conclusion, the LN freezing technique can achieve reliable local control in treating proximal tibial sarcoma. The patella cryo-free technique preserves the biological properties of the resected patella and enhances fracture healing. Compared with other methods, including tumor prosthesis and traditional biological reconstruction for proximal tibial malignancy, we believe



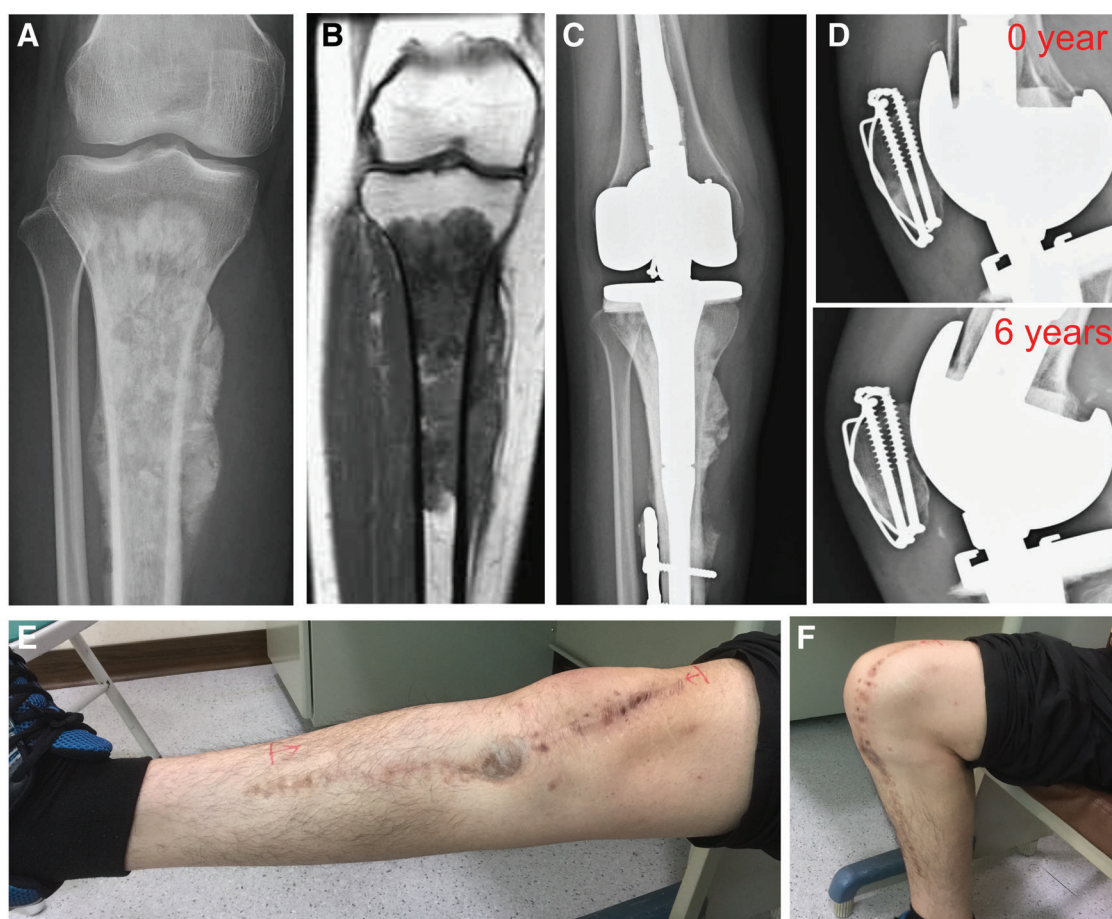
**Table 2****Clinical result and functional performance**

	Group A (patella freezing, n = 13)	Group B (patella cryo-free, n = 10)	<i>p</i>
Follow-up			0.248 <sup>b</sup>
Months (mean ± SD)	79.2 ± 22.2	77.1 ± 19.1	
Q1-Q3	66.0-97.0	66.8-88.5	
Local recurrence	0	0	
Lung and distal metastasis	3 (3 expired)	2 (1 expired)	0.859 <sup>a</sup>
Tibia graft-host nonunion	0/10	0/9	
Patellar complication	8	1	0.012 <sup>a</sup>
Resorption	3	0	
Nonunion/malunion	5	1	
Extension lag	21.2 ± 7.2	6.0 ± 5.2	<0.001 <sup>b</sup>
° (mean ± SD) (Q1-Q3)	15.0-25.0	1.3-10.0	
Flexion	98.7 ± 6.2	113.5 ± 6.3	<0.001 <sup>b</sup>
° (mean ± SD) (Q1-Q3)	95.0-105.0	110.0-115.0	
MSTS score	82.56 ± 5.64	93.33 ± 3.85	0.001 <sup>b</sup>
% (mean ± SD) (Q1-Q3)	80.00-86.67	90.00-93.33	
Complication	3 (superficial flap complication)	1 (superficial flap complication)	0.412 <sup>a</sup>

MSTS = Musculoskeletal Tumor Society.

<sup>a</sup>χ<sup>2</sup> test.<sup>b</sup>Mann-Whitney U test, two-tailed.

**Fig. 6** A-F, A 52-year-old male patient with an undifferentiated pleomorphic sarcoma of the left proximal tibia who was treated with traditional patella freezing method and recycled frozen bone-prosthesis composite reconstruction. A, Preoperative plain radiograph after neoadjuvant therapy shows intact bone integrity. B, Preoperative MRI study shows the border of the tumor. C, Plain radiograph at postoperative 6.5-year follow-up shows complete union of the host-graft junction. D, Lateral radiograph reveals patellar malunion, migration of the screw, and resorption of the lower pole of the patella. E and F, Functional performance at the last follow-up shows 15° of extension lag. The Flexion was only 90° and the Musculoskeletal Tumor Society score was 83.33%. MRI = magnetic resonance imaging.



**Fig. 7** A-F, A 17-year-old male patient with an osteosarcoma of the right proximal tibia who was treated with patella cryo-free method and recycled frozen bone-prosthesis composite reconstruction. A, Preoperative plain radiograph after neoadjuvant therapy shows intact bone integrity. B, Preoperative MRI study shows the border of the tumor. C, Plain radiograph at postoperative 6-year follow-up showing complete union of the host-graft junction. D, Lateral radiograph revealing complete union of the patella and no implant migration after the operation. E and F, Functional performance at the last follow-up. The patient achieved full extension of the knee joint without any lag. The knee joint flexion is  $107^\circ$  and the MSTS score is 93.33%. MRI = magnetic resonance imaging.

the application of patella cryo-free technique in frozen recycled autograft preserves the biological property of the resected patella, reduces patellar nonunion or malunion rates, and provide better clinical outcomes.

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