Dear Reviewer:

Thank you very much for your suggestion. I tried to correct according to your comments.

Red colored area is added sentences.

1. The application of tissue engineering and stem cells in this field is a hot research topic. There are many important research reports and progress. It is recommended that the author fully elaborate on this part to help readers understand the current research progress and the main problems.

Answer: I added the sentences of **Biological augmentation of ACL repair and reconstruction** “The four main components of tissue engineering such as cells, growth factors, scaffolds, and mechanical stimuli, are combined using various methods of bioaugmentation. They have been increasingly explored to improve outcomes after surgical treatment of ACL injury [167-171].

**Scaffolds:** Stem cell-based tissue regeneration combined with scaffolds represent a novel treatment for torn ligaments [172-174]. Three-dimensional (3D) scaffolds seeded with mesenchymal stem cells yielded excellent results in osteointegration enhancement between the tendon and bone tunnel in ACL reconstruction with a rabbit model [175]. Platelet-rich plasma (PRP) combined with a gelatin sponge to prolong PRP bioactivity promotes mesenchymal stem cell proliferation in vitro [176].

**Cell sources:** The main cell sources are mesenchymal stem cells and ACL fibroblasts [177]. Mesenchymal stem cells have higher proliferation and collagen production rates than ligament fibroblasts [178]. ACL-derived human-induced pluripotent stem cells might be a promising cell source for ligaments and related tissue engineering applications [179].

**Growth factors:** PRP is obtained by plasma separation. PRP contains platelets, blood proteins such as fibrin, and a mixture of growth factors such as platelet derived growth
factor (PDGF), insulin-like growth factor (IGF), vascular endothelial growth factor (VEGF), and transforming growth factor-beta (TGF-beta), which are involved in general healing processes.

PRP has been used to treat knee OA and to promote ligament healing. Recently, it has been used experimentally in ACL reconstruction to promote graft maturation and osteointegration \cite{180}. However, no clinical efficacy data have been reported yet \cite{181,182}.

**Mechanical stimulation**: Mechanical stimuli and dynamic loading are necessary for ligaments to enhance matrix synthesis and maintain their strength \cite{183}. Electrospinning has been effective for cell proliferation and extracellular matrix production of scaffolds for ligament tissue engineering \cite{184}. However, whether any mechanical stimulation is required to implant tissue-engineered ACL constructs is controversial \cite{177}.

In recent studies, bioenhanced ACL repair had similar results as ACL reconstruction. These biotherapies are expected to reduce postsurgical osteoarthritis and to be improved in the future.”.

2. Complications of ACL are an important issue of concern to surgeons. The authors should elaborate various complications and accidents in the operation, and discuss the prevention methods and principles of treatment.

Answer: I added the sentences of complication **“Deep vein thrombosis**: The incidence of deep vein thrombosis after ACL reconstruction ranges from 0.3\% \cite{129} to 0.4\% \cite{130}. The incidence of pulmonary embolism is 0.18\% \cite{129} to 0.046\% \cite{130}. The only significant risk factor is age. Therefore, thromboprophylaxis should be considered in older patients.

**Hemarthrosis**: Hemarthrosis after ACL reconstruction can delay rehabilitation. The use of intravenous tranexamic acid in ACL reconstruction results in reduced joint drain
output and hemarthrosis as well as less pain and greater range of motion during the early postoperative period \textsuperscript{[131]}. Tranexamic acid does not increase the risk of deep vein thrombosis after surgery \textsuperscript{[132]}. 

**Joint stiffness:** The incidence of joint stiffness after ACL reconstruction is overall 3\% \textsuperscript{[133]} to 8.8\% \textsuperscript{[134]}. There was no significant difference between BTB graft and hamstring tendon with respect to the frequency \textsuperscript{[134]} and the interval between trauma to surgery \textsuperscript{[133]}. 

Cyclops syndrome after ACL reconstruction is due to a fibrous nodule in the anterior part of the intercondylar notch. It restricts the full extension of the knee \textsuperscript{[135]}. The incidence of symptomatic cyclops syndrome ranges from 1.9\% to 10.9\% \textsuperscript{[135]}. 

Arthrofibrosis is a rare but potentially devastating complication after ACL reconstruction \textsuperscript{[136]}. Approximately 2\% of patients have postoperative stiffness that requires intervention \textsuperscript{[137]}. However, arthrofibrosis remains poorly defined and there are no clear treatment guidelines \textsuperscript{[138]}. 

**Nerve injury:** Tendon harvesting for ACL reconstruction often injures sensory branches of the saphenous nerve \textsuperscript{[139]}. Injuries to the sartorial branch of the saphenous nerve associated with medial incisions for hamstring tendon harvesting are more common than injuries to the infrapatellar branch associated with midline incisions for patellar tendon harvesting \textsuperscript{[139]}. Numbness of the skin surface supplied by the infrapatellar branches of the saphenous nerve (IPBSN) after ACL reconstruction are less common with the quadriceps tendon compared with the hamstring tendon \textsuperscript{[140]}. Regarding hamstring tendon harvesting for ACL reconstruction, vertical incisions increase the risk of iatrogenic injury to the IPBNS compared with oblique incisions \textsuperscript{[141-144]}. 

**Patellar fracture:** The incidence of patellar fracture during BTB harvesting ranges
from 0.3% \[^{[145]}\] to 1.3% \[^{[146]}\]. It is a rare but serious complication \[^{[147]}\]. To eliminate the risk of perioperative patellar fracture, the bone-tendon-autograft technique, which does not harvest the inferior patellar bone, might be an alternative graft option \[^{[148]}\].

The incidence of intraoperative patellar fracture after harvest of a quadriceps tendon autograft is reported to be 3.5%. It is necessary to use care when harvesting the bone block from a central position \[^{[149]}\] and to limit the depth of bone harvesting to less than 50% of the depth of the patella with a shorter bone plug length. Longitudinal cuts can be angled centrally to produce a trapezoidal bone block with shallower bone removal \[^{[149]}\].

3. The main points of various surgical methods are too simple. The author should elaborate them one by one, so as to be of reference value to readers.

Answer: I added the Table 1 and Preparation “Hamstrings: The hamstring tendon is harvested by making a 3–4 cm skin incision 2 cm distal to the medial tibial articular surface. First, the sartorius tendon is divided along the length of the tendon. The underlying gracilis tendon is confirmed and pulled proximally. The semitendinosus tendon is confirmed distally. The distal branch of the semitendinosus tendon is dissected, bluntly detached with forceps and a gauze ball, and collected using a tendon stripper. If the semitendinosus tendon is short or thin, the gracilis tendon is also harvested.

**BTB:** The bone–patellar tendon–bone (BTB) graft is harvested by making a longitudinal skin incision of approximately 5 cm along the medial edge of the patellar tendon. An incision is made in the central 9–10 mm of the width of the patellar tendon with a scalpel. Trapezoidal bone fragments with a width of 8–10 mm and a length of 15 mm are collected from the patella and tibial tuberosity. In a BTB graft, when the length
of the tibial tunnel is short and the patellar tendon is long, the bone fragment on the tibial side is exposed outside of the tibial bone tunnel. Thus, it is necessary to prepare a fixture such as a post screw.

**Quadriceps**: The surface layer of the quadriceps is the rectus femoris tendon. The middle layer consists of the tendons of the vastus medialis and vastus lateralis muscles. The deep layer is the vastus intermedius tendon. The width is narrowest approximately 5 cm proximal to the patellar attachment \[14\]. To harvest the quadriceps tendon, a scalpel is used to make a full-thickness incision 5–6 mm from the patellar attachment to the proximal end of harvested tendon. Next, an incision is extended up to the patellar attachment along the length of the tendon. The width of adherent portion is 8–10 mm. Next, a tendon with a thickness of approximately 10 mm is harvested. A Krackow suture is performed with two sets of no. 2 sutures. The length of the grafted tendon can be easily adjusted.

On the patellar side, a trapezoidal bone fragment with a length of 15 mm and a width of 8–10 mm is harvested. Even if the width of the quadriceps tendon is 5–6 mm, the thickness (approximately 10 mm) is sufficient in combination with the rectus femoris, vastus medialis, vastus lateralis, and vastus intermedius. The cross-sectional area of the quadriceps tendon is almost twice that of the BTB graft. The quadriceps tendon has higher load to failure and stiffness than the BTB graft \[15\]. The quadriceps tendons may be easier to use than the BTB graft in patients with anterior knee pain and pain during kneeling \[16\]. To fix the grafted ligament, an interference screw or a cortical button such as the CL-BTB is used as a patellar fragment on the femoral side. The thread is tied tightly to the cortical button on the tibial side.”

I added the sentences of Techniques for femoral tunnel drilling “Anteromedial
**Technique**: The arthroscope is inserted from the anterolateral portal with the knee bent to 120–130 degrees. The drill guide pin is inserted from the anteromedial portal. The pin tip is placed in the center of the femoral footprint of the ACL. After drilling, it penetrates from the lateral cortex to the skin surface. First, a PL bundle bone tunnel is made, followed by an AM bundle bone tunnel in the same manner. If the knee flexion angle is 120 degrees or less, blowout of the posterolateral cortex would occur [40, 41, 42], making it impossible to fix the femoral side; this increases the risk of peroneal nerve palsy.

**Outside-in technique**: The arthroscope is inserted from the anteromedial portal with the knee bent to approximately 90 degrees. The femoral drill guide is inserted into the joint from the anterolateral portal and held firmly in place. Next, the guide pin is inserted so that it does not shift from the center of the femoral footprint of the ACL. The drill-guided trocar should be placed on the femur. A skin incision should be made in the lateral thigh to locate the lateral cortex.

With the anteromedial and outside-in approaches, after creating the femoral bone tunnel, a tibial drill guide is used to create a tibial bone tunnel from the anterior surface of the tibia based on the bone tunnel diameter.”

I added the sentence of Transtibial technique: “An arthroscope is inserted from the anterolateral portal. A tibial drill guide is inserted into the joint from the anteromedial portal. The tip of the guide is applied to the footprint of the tibia”

I added the sentence of Double-bundle or single-bundle ACL reconstruction “A double bundle consists of two routes: AM and PL bundles. Two bone tunnels are created for the femur and tibia, respectively. The graft material consists of hamstring autograft or allograft. In most cases, a hamstring autograft is used. The anteromedial approach is
often used to create bone tunnels, but the transtibial or outside-in approach may be used.

An arthroscopic ruler can be used to measure the insertion site of the patient’s native ACL. This measurement can help decide whether to perform double- or single-bundle reconstruction. When a patient has femoral and tibial insertion sites that are larger than 14 mm, double-bundle reconstruction is indicated. When a patient has a notch width of less than 12 mm, double-bundle reconstruction often cannot be performed because the AM guide pin cannot be placed in the native femoral insertion site.”

4. After the elaboration of each surgical method, the author should put forward the indications and operative points of each surgical method based on his own clinical experience.

5) Answer: I added the Table 1 and added the sentence “Our methods for ACL reconstruction: We usually use only the semitendinosus tendon for primary ACL reconstruction. We produce the bone tunnel with the all-inside method using a single quadruped semitendinosus graft. We fix the grafted ligament with a cortical suspension button on both sides. In the all-inside method, the knee is flexed to approximately 90 degrees, the lower leg is internally rotated, and varus stress is applied to the knee using the dependent method.

We developed a tibial drill guide with a laser beam that can identify the optimal location for the femoral tunnel during creation of tibial tunnel in a modified transtibial method. We used it in a clinical application during ACL reconstruction. The new drill guide system: The structure of the tibial drill guide with a laser beam is shown in Fig. 2. This laser beam-guided technique with a special tibial drill guide produces both tibial and femoral tunnels. The laser pointer was visible light semiconductor laser, maximum output energy of 1mW. The guide contains a metal tube for passage of laser beam (Fig. 3), which can be filled with saline for irrigation. The reflected beam indicates
appropriate position on the extension of the pin (Fig. 4). Fig. 5 shows an arthroscopic photography with the laser beam. **Transtibial guide pin placement and tunnel placement:** The special drill guide is inserted through the anteromedial portal, and placed at the anatomical tibial foot print. A laser beam is reflected by reflecting plate of tip of the guide. The laser pointer illuminates the tunnel which is where femoral bundle should be made appropriately (Fig. 4). A transtibial guide pin of 2.4 mm in diameter is inserted into the intra-articular portion of proximal tibia. The diameter of tibial tunnel is similar to that of grafted tendon. The guide pin is set at appropriate location of femoral tunnel. Method of making femoral tunnel and graft fixation was performed according to our previously described [56, 75]. Our method is a useful way to select an appropriate anatomical site for the bone tunnels accurately and obtain excellent clinical results with ACL reconstruction.

Recently, we have produced and used a drill guide for the all-inside transfemoral method. The grafted ligaments are fixed with a CL-BTB endobutton on the femoral side and a cortical button on the tibial side with knee flexion of approximately 20 degrees. We often use a BTB graft to obtain strong fixation for young men who are active athletes needing to withstand strong collisions, such as in rugby and football. However, the quadriceps tendon is also useful. Residual ligaments are often preserved if they are thin but relatively tense. At this time, double-bundle reconstruction with residual ligaments is not performed because it is difficult to make two bone tunnels at appropriate positions. We reconstruct the AM or PL bundle based on preoperative MRI evaluation and intraoperative arthroscopic findings. ACL reinforcement is often performed to reconstruct the PL bundle, which can lead to definite symptoms of rotatory instability.
In revision reconstructive surgery, we use the ipsilateral semitendinosus tendon, BTB graft, or quadriceps tendon with a patellar fragment, unless the tendon has already been used. Quadriceps tendon with a patellar fragment has excellent mechanical strength. Reconstruction can be performed with the all-inside method, which reduces trabecular damage in the bone tunnel. Thus, we plan to increase its use in the future. At this time, the grafted ligament is fixed with a patellar fragment on the femoral side using an interference screw. On the tibial side, the grafted ligament is fixed with a cortical button, which is sutured using the Krackow method with two sets of no. 2 sutures.”

And I added the figure 2-5 regarding our surgical procedure.

5. Computer-Aided ACL reconstruction system includes a variety of techniques, including surgical planning, navigation, template, preformed implant, etc. The author only expounds the navigation, and other contents should be added.

Answer: I added the sentence of Computer-aided surgery “There are four main types of applications for navigation systems in ACL reconstruction [191, 192]: (1) technical assistance of tunnel placement for tibial or femoral tunnel drilling; (2) kinematic evaluation to analyze the biomechanical behavior of the ACL and surrounding structures during reconstructive surgery [193]; (3) comparison of the effectiveness of different surgical techniques for making laxity measurements [194]; and (4) navigation to improve clinical outcomes and cost-effectiveness of ACL reconstruction.

3D fluoroscopy-based navigation system [195, 196]

It is essential to perform preoperative planning using 3D CT images before operation. A reference frame is rigidly attached to the femur with two half-pins at the beginning of surgery. An intraoperative 3D image of the distal femur is obtained with the C-arm of
the image intensifier, which is equipped with a wireless tracker. The image is reconstructed into a 3D image on the computer screen. A navigation computer helps the surgeon visualize the entire area for bone tunnel creation. However, this system requires fixation with two half-pins in the lateral femur, which necessitates an additional skin incision and more drill holes.

**CT-based navigation without intraoperative fluoroscopy** [197-199]

This system uses a preoperatively generated 3D model from CT images or intraoperative 3D bone morphing with an optical tracking system. The optical tracking system captures reference markers that are rigidly attached to the patient and surgical tools. After fixing the tracking markers, approximately 20 landmark points are collected on the surface of the bone with probes.

Anatomical reconstruction using the anteromedial technique is associated with more risks including (1) a short femoral tunnel, (2) posterior wall blowout, and (3) iatrogenic damage to the cartilage of the medial femoral condyle due to the more horizontal direction of the femoral tunnel in the 3D plane [41]. Navigation systems with enhanced registration accuracy can reduce surgical failures such as short femoral tunnels and posterior wall breakage of the distal femur [197, 200].

**Image free navigation system**

This method does not require preoperative CT or intraoperative fluoroscopy. The transmitters for the femur and tibia are fixed with pins to register intra- and extra-articular landmarks intraoperatively. Next, the transmitter is attached to the tibial drill guide to determine the location of the tibial bone tunnel. The same maneuver is used for the femoral bone tunnel [201].

There is considerable variability in intra-articular landmark identification with
There is a potential risk of miscalculating tunnel positions [202]. Guided drilling of the tunnel leads to errors as small as 2.5 mm in the footprint and in the orientation of the intra-operative video for guiding the drilling of the tunnel with a set of contours which is reconstructed by touching the bone surface with an instrumented tool [203].

There are some studies on the use of computer-assisted navigation for bone tunnel positioning and evaluating joint instability in ACL reconstruction [204-206]. Clinical, radiological, and functional comparisons between computer-assisted and conventional ACL reconstruction have found increased accuracy in femoral tunnel placement with the use of navigation systems compared with traditional techniques alone [198, 207-210]. Some studies reported that computer-assisted navigation improved the accuracy of tunnel positioning [211-213]. For inexperienced surgeons, navigation systems could be useful in ACL surgery to avoid malpositioning of bone tunnels [214, 215].”

6. Artificial substitution material is another important direction in this field, which should be supplemented by the author.

Answer: I added the sentence of Biological augmentation of ACL repair and reconstruction “Scaffolds: Stem cell-based tissue regeneration combined with scaffolds represent a novel treatment for torn ligaments [172-174]. Three-dimensional (3D) scaffolds seeded with mesenchymal stem cells yielded excellent results in osteointegration enhancement between the tendon and bone tunnel in ACL reconstruction with a rabbit model [175]. Platelet-rich plasma (PRP) combined with a gelatin sponge to prolong PRP bioactivity promotes mesenchymal stem cell proliferation in vitro [176].”
I send the reference of 225, however, uploaded reference is 228. I could not change the number. Thank you for your kindness.

Sincerely yours.

Toshiaki Takahashi, Ehime university, Japan.