Chapter 42
Strategies to Enhance Biological Tendon-Bone Healing in Anterior Cruciate Ligament Reconstruction

Tomoyuki Matsumoto and Ryosuke Kuroda

Abstract Tissue engineering techniques to enhance tendon-bone healing in anterior cruciate ligament (ACL) reconstruction, including stem cells and growth factors/cytokines, are gaining wide acceptance, and their clinical feasibility has also been recognized. Among them, vascular stem cells at the site of ruptured ACL, which have high proliferation and multi-differentiation potential, accelerate tendon-bone healing by enhancing angiogenesis and osteogenesis in human-rat xenotransplantation and canine autologous transplantation model of ACL reconstruction. A pilot clinical study, which used ruptured tissue for ACL reconstruction, indicated reduction of tunnel enlargement despite no improvement in clinical scores. However, for effective clinical application in future, detailed analysis is required regarding enrolled patient demographic parameters, such as age, sex, surgical timing, and type of ACL injury. This chapter highlights effectiveness of vascular stem cells application for early tendon-bone healing in ACL reconstruction, providing an insight for future strategies.

Keywords Tendon-bone healing • Stem cells • Ruptured tissue • Angiogenesis • Osteogenesis

42.1 Introduction

When an anterior cruciate ligament (ACL) is ruptured, the healing potential is considered to be extremely poor [1, 2]. Therefore, ACL reconstruction has become fairly standardized, with clinical success rates of 70–95 % [3–5]. Anatomical double-bundle (DB) reconstruction procedures using hamstring grafts have recently become widespread with promising results [6–9]. Whereas most surgical procedures in this area require healing and maturation of tendon grafts in a surgically

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created bone tunnel, the attachment between the tendon and the bone is the weakest region in the early posttransplantation period [10, 11]. In fact, mechanical properties of the healing ligament did not return to normal 1 year after injury in both rabbit and canine models [12, 13]. Therefore, secure fixation of the tendon graft to the bone is a significant factor in allowing earlier and more aggressive rehabilitation and earlier return to sports and work.

Current treatment with hamstring grafts has achieved satisfactory anteroposterior and rotational stability but can cause significant tunnel enlargement [14, 15]. Tunnel widening is believed to be multifactorial in origin. Some mechanical causes are graft motion [15] and stress deviation inside the tunnel and inappropriate location of the graft and tunnel [16]. From a biological aspect, poor healing potential at the tendon-bone interface also results in tunnel enlargement. Large tunnels often require revision ACL surgery and necessitate staged procedures [17]. Therefore, enhancing tendon-bone healing and preventing bone tunnel enlargement are closely related and, therefore, vital in ACL reconstruction.

Tissue engineering techniques with stem cells or growth factors/cytokines have been explored to achieve early healing and better tendon-bone integration [18]. Several animal studies focused on enhancement of tendon-bone healing in ACL reconstruction used periosteum [19, 20], bone marrow stromal cells [21], bone marrow mesenchymal stem cells (MSCs) [20, 22], injectable tricalcium phosphate [23], and other growth factors [24–28]. Although these biological engineering strategies are currently experimental, they are expected to be used in clinical setting in the near future.

42.2 Blood Vessels as a Potential Target for Tendon-Bone Healing in ACL Reconstruction

Over the last decade, there have been considerable controversies regarding the ACL's intrinsic healing potential. Some surgeons are of the view that the ACL does not heal without reconstruction due to the lack of blood clot formation, insufficient vascular supply, deficits in intrinsic cell migration, impaired growth factor ability, and effects of synovial fluid on cell morphology [29, 30]. On the other hand, others have reported that the ACL spontaneously heals without surgery [31–33], or only with primary sutures [34–37]. In fact, during acute and subacute arthroscopic procedures for ACL reconstruction, a tibial stump is often visualized that can have connecting fibers to the femur and the tibia or between the posterior cruciate ligament and tibia, suggesting healing potential in ACL fibers. However, there is no scientific evidence till now.

Stem cells' qualities of high expansion, self-renewal, and multi-differentiation present a reasonable explanation for the healing potential of the ACL. Although some findings show the existence of MSC-like cells in human ACL tissues [38, 39], their origin and characteristics still remain unclear. Recently, blood vessels have

been reported to be a richer supply of stem/progenitor cells with expression of CD34 and CD146 surface cell marker [40–43].

Matsumoto et al. demonstrated the presence in subacutely ruptured ACL tissues of CD34-expressing vascular cells with potential for multi-lineage differentiation that can be recruited to the ACL rupture site to support healing [44]. They confirmed the rich vascularity in the ruptured site and septum region when compared with mid-substance using H&E and immunohistochemical vascular staining. In addition, using immunohistochemistry and flow cytometry analysis, they confirmed recruitment of CD34+ and CD146+ cells with multi-lineage differentiation potential to the ruptured site when compared with the gathered cells as the mid-substance (Fig. 42.1a). These cells demonstrated multi-lineage differentiation potential including osteogenesis, adipogenesis, chondrogenesis, and endotheliogenesis (Fig. 42.1b). Covas et al. recently discovered that MSCs and pericytes are similar cells located in the vasculature wall, and they function as cell sources for repair and tissue maintenance [40, 45]. Findings reveal that CD34+ cells are committed not only to endothelial cells but also mural perivascular cells (i.e., pericytes and smooth muscle cells) [46, 47]. Similarly, vascular pericytes with CD146 expression may arise from CD34+ cells [41]. Furthermore, Zengin et al. reported the existence of endothelial progenitor cells and stem cells in a distinct zone between the smooth muscle and the adventitial layer of human adult vascular wall that are capable to differentiate among mature endothelial cells and hematopoietic and local immune cells, such as macrophages [43]. Based on these findings, CD34+ cells with high expansion and multi-differentiation potential in the ACL ruptured site, which were converted into cell population positive for CD146, CD44, CD90, and CD73 expression [44], may have similar characteristics of MSCs described over the last decade [48] and have a possibility to provide an attractive cell source for tissue repair and regeneration.

Among multi-lineage differentiation potentials, osteogenic and endothelial differentiations are especially important for ligament or tendon-bone healing. There are some reports concerning osteogenesis and angiogenesis/vasculogenesis for ligament or tendon-bone healing. To accelerate osteogenesis and/or angiogenesis for tendon-bone healing, vascular endothelial growth factor (VEGF), granulocyte colony-stimulating factor (G-CSF), transforming growth factor-β (TGF-β), bone morphogenetic protein 2 (BMP2), and BMP7 have recently received attention for their therapeutic potential [27, 28, 49–51]. However, Tei et al. reported that human G-CSF-mobilized peripheral blood CD34+ cells contribute to ligament healing via their endothelial differentiation (vasculogenesis) and enhanced intrinsic angiogenesis by VEGF secretion in a immunodeficient rat model [52]. In addition, Matsumoto et al. showed that peripheral blood CD34+ cells could be differentiated into osteoblasts and endothelial cells in a fracture model [53, 54]. Ratio of CD34+ cells is only 1 % in the peripheral blood cells [53] compared to 44 % [44] in ACL ruptured tissue cells, suggesting that isolation of CD34+ cells from the ACL tissue is less important than that from peripheral blood.

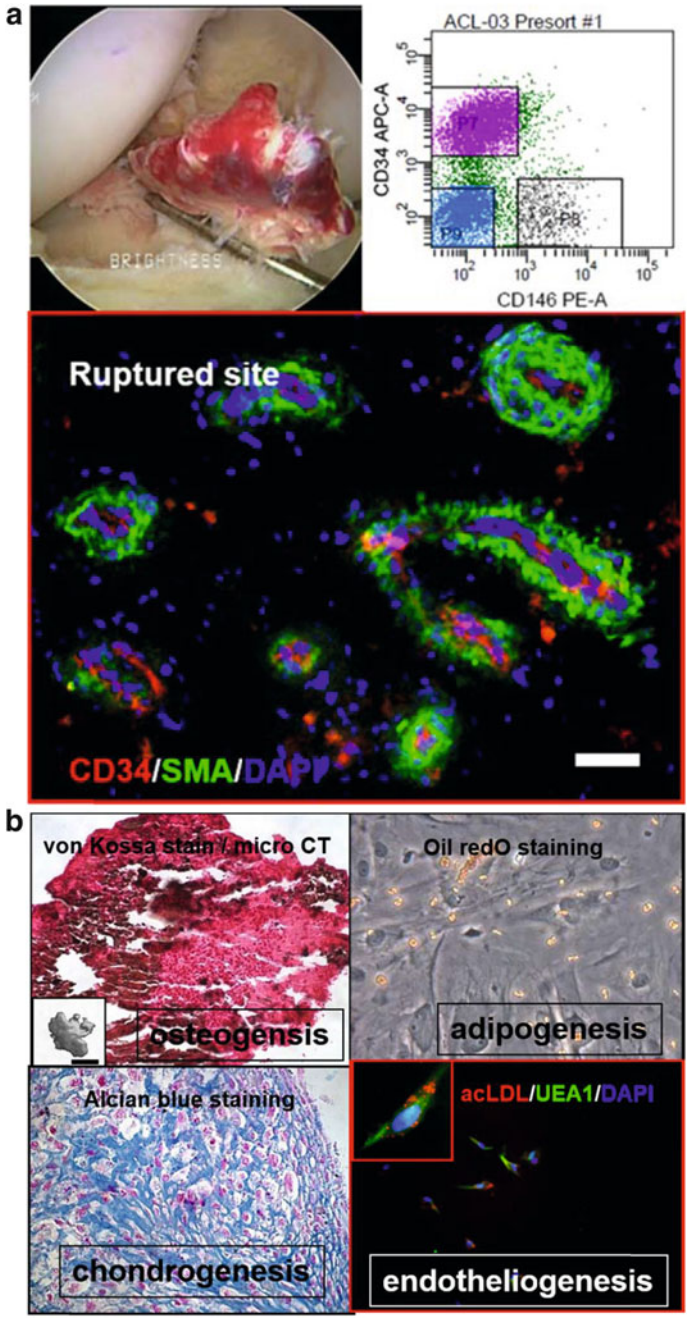

Fig. 42.1 In vitro experiments showing vascular stem cells in the ACL ruptured tissue (**a**) Tissues showing more positive staining for CD34 in the ruptured site than in the mid-substance (**b**) CD34-positive cells from ACL ruptured tissue showing multi-lineage differentiation potential including osteogenesis, adipogenesis, chondrogenesis, and endotheliogenesis

42.3 Therapeutic Potential of ACL-Derived Vascular Stem Cells or Ruptured Tissue for Tendon-Bone Healing in ACL Reconstruction

Based on the report showing the existence of CD34+ vascular stem cells in ACL ruptured tissue [44], Mifune Y et al. demonstrated that intracapsular transplantation of human ACL-derived CD34+ cells from the ruptured site contributed to tendon-bone healing via angiogenesis/vasculogenesis and osteogenesis in an immunodeficient rat model of ACL reconstruction [55]. Using a molecular approach, they confirmed enhanced intrinsic angiogenesis/osteogenesis and human-derived vasculogenesis/osteogenesis by intracapsular transplantation of human ACL-derived cells. Histological, radiological (CT), and biomechanical assessment exhibited early tendon-bone healing by cell transplantation. Nonselected as well as CD34+ cells contributed to tendon-bone healing and reduction of tunnel enlargement in a rat model of ACL reconstruction.

During cell therapy for ACL reconstruction, second-step arthroscopic surgery is unavoidable due to the necessity of cell isolation, cell culture, and cell expansion, thus affecting the clinical feasibility of CD34+ cell transplantation. Based on the rich supply of CD34+ cells in the ACL ruptured site [44] and effectiveness of nonselected cells in a rat ACL reconstruction model [55], Matsumoto et al. explored the effect of ACL ruptured tissue on tendon-bone healing in ACL reconstruction. To explore the feasibility of the use of ruptured tissue in the clinical setting, the study was designed as an autologous transplantation model with a large animal canine [56]. ACL ruptured tissue was harvested 2 days after ACL resection and was sutured to the grafts in the tibial tunnel in ACL reconstruction (Fig. 42.2a). The results in histological, CT, and biomechanical testing showed early tendon-bone healing and reduction of tunnel enlargement compared to control group (no tissue suture) (Fig. 42.2b). These findings may lead to the effectiveness of ruptured tissue in ACL reconstruction in the clinical application.

42.4 Clinical Application of ACL Ruptured Tissue in ACL Reconstruction

Based on previous findings, Matsumoto and Kuroda et al. compare 2-year clinical outcomes and tunnel enlargement of DB ACL reconstruction with and without suturing of the autologous ruptured tissue to the grafts in patients with subacute ACL injury (Fig. 42.3) [57]. In this study, 10 patients with subacute (within 3 months after injury) ACL rupture were randomly allocated to undergo DB ACL reconstruction with suturing of the ruptured tissue to hamstring grafts or conventional DB ACL reconstruction in two equal control groups ($n = 5$ each). The results showed significant reduction in tunnel enlargement as assessed with 3D-MDCT in the tissue group, especially at the femoral side. However, the postoperative Lysholm score, anterior

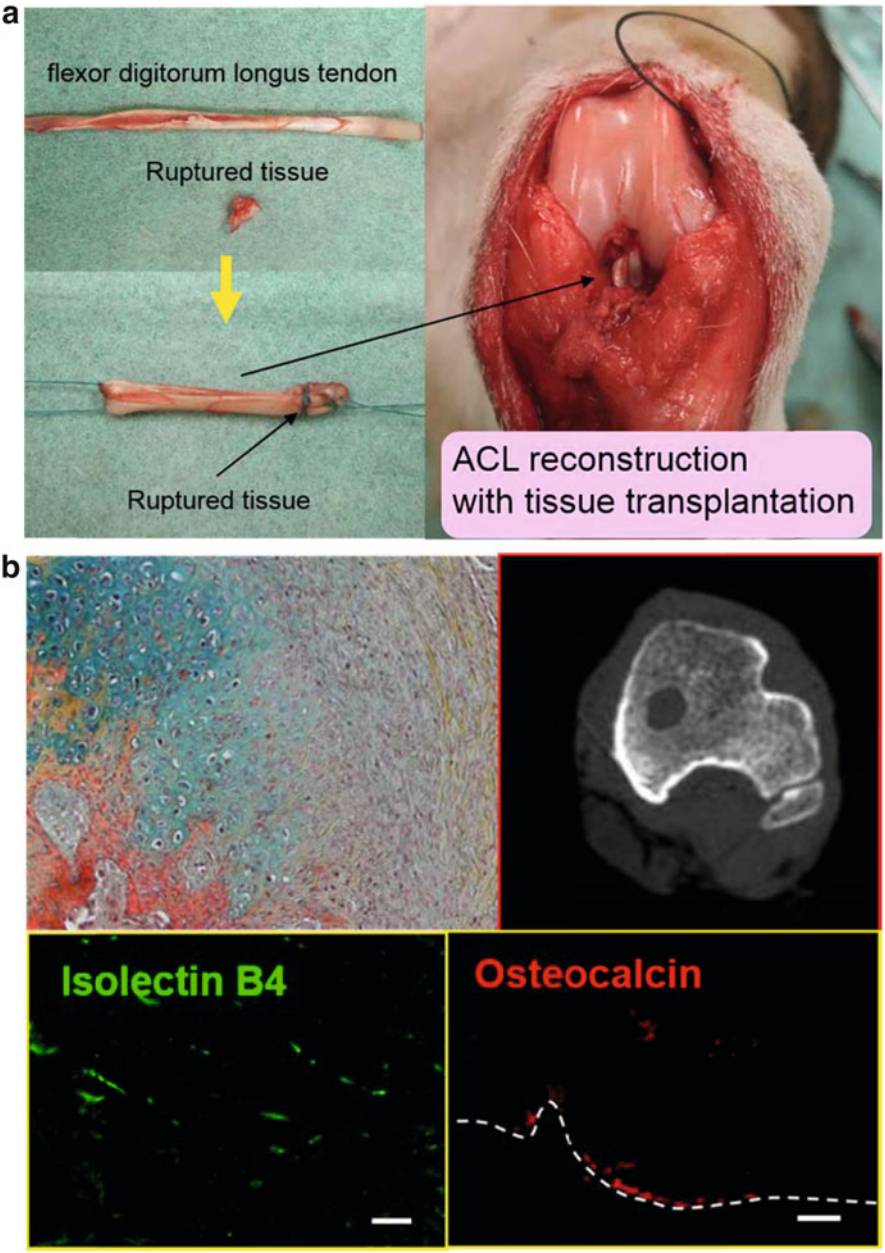

Fig. 42.2 Preclinical study using canine ACL reconstruction model
(**a**) ACL reconstruction was performed using tendon graft with ruptured tissue
(**b**) Autologous tissue transplantation exhibited early tendon-bone healing and bone tunnel reduction via enhanced angiogenesis and osteogenesis

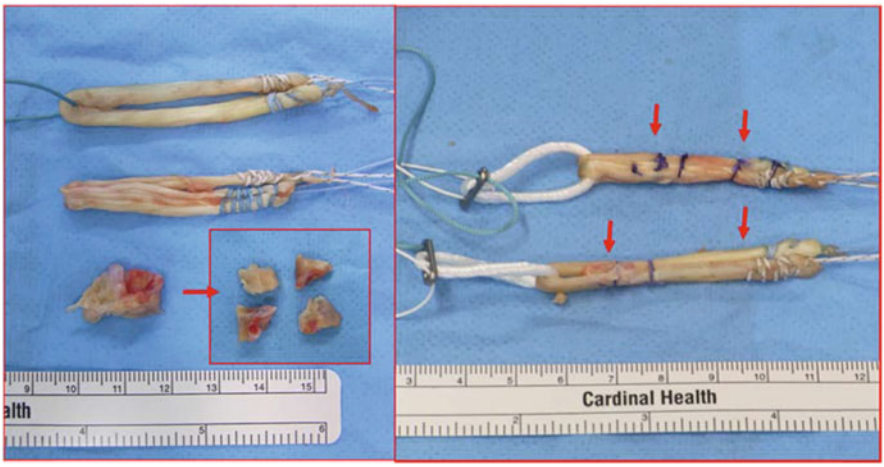

Fig. 42.3 A pilot clinical study using ruptured tissue in ACL reconstruction surgery
ACL reconstruction was performed with the use of ruptured tissues which were sutured to the grafts located in the tunnels

stability of the knee measured with the KT-1000 arthrometer, and rate of negative manual pivot shift test did not differ significantly between the two groups. In several animal studies, the use of periosteum [19, 20], bone marrow mesenchymal stem cells [20, 22], injectable tricalcium phosphate [23], and growth factor [26–28] was reported to enhance tendon-bone healing in ACL reconstruction. Among those, application for human ACL reconstruction was only limited to periosteum with promising results [58–60]. Chen CH et al. reported after their 2–7-year clinical follow-up in 312 patients that satisfactory results could be achieved with the periosteum-enveloping hamstring tendon graft in single-bundle ACL reconstruction with minimal tunnel widening (more than 1 mm tunnel widening: 5.4 % of femoral and 6.1 % of tibial side). Considering this comparison, concept for the treatment is similar and successful tunnel reduction was found on radiographs [58]. If the strategy using ruptured tissue has advantages over previous strategies for enhancement of tendon-bone healing, the ruptured ACL tissue can be used with easy clinical settings without any additional incision, procedure, and cell isolation and expansion.

Preservation of the remnant ACL reconstruction has recently received attention focused on the existence of mechanoreceptors in the ACL remnant that contribute to the proprioceptive function of the ACL [61–65]. However, the intrinsic healing potential of ACL remnants after ACL reconstruction has not been fully investigated. In the pilot study based on a previous series [44, 55, 56], the rupture site of the ACL remnant was harvested and transplanted to the grafts to augment healing, especially at the tendon-bone integration site. This technique is reliable, simple, surgeon-friendly, and inexpensive, and thus clinically feasible.

To predict outcomes of ACL reconstruction surgery, the characteristics of patients should be considered. Uefuji et al. recently reported that ruptured ACL

remnants have a healing potential with multi-lineage differentiation, including osteogenesis and endotheliogenesis; however, this potential is age dependent and decreases with age, as CD34+ cells were more prevalent in the ACL remnants in younger patients [66]. ACL remnants in younger patients exhibited high proliferation and great multi-lineage differentiation potential, especially in osteogenic and endothelial differentiation. Furthermore, with the use of in vivo rat ACL reconstruction model, Nakano et al. reported that the healing potential of human ACL-derived cells on the maturation of tendon-bone healing is dependent on the patient's age [67]. Considering these evidences, patient age can be one of the factors that influence postoperative outcomes in healing potential for ACL-derived cells or remnant. In the near future, other demographic factors such as interval between injury and surgery, sex, type of injury, and patient activity level should be assessed to explore other factors that impact ACL remnant-derived cells in the healing potential of reconstructed ACL.

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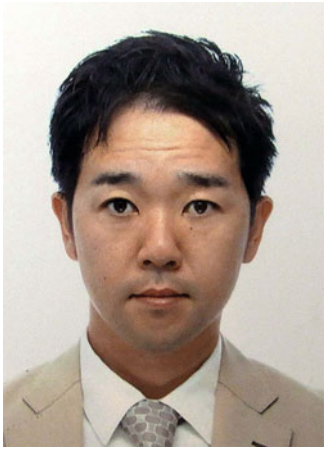

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