

An Overview of the Facilitation of Statins to Tendon-bone Healing after Anterior Cruciate Ligament Reconstruction

Junxi Huang

*International School, Jinan University, Guangzhou, China
hix459@stu2022.jnu.edu.cn*

Abstract: The Anterior cruciate ligament (ACL) helps stabilize the knee joint and support knee movement. When it is torn severely, ACL reconstruction is recommended to patients and after the surgery, tendon-bone healing becomes a problem to face for patients' rehabilitation. Statins are usually prescribed as lipid-lowering drugs in clinics, while some studies have revealed that statins have an influence on promoting the tendon-bone healing process. It aims to conclude the promoting ability of statins on tendon-bone healing after ACL reconstruction and provide evidence for future research and clinical application in the field based on existing literature and relevant data. Statins can promote the process of tendon-bone healing in ACL reconstruction both histologically and biomechanically under an appropriate concentration. Studies on relevant fields gave support to the effectiveness of statins in enhancing the healing process at the tendon-bone interface. Moreover, this benefit can be improved when statins are locally administered by approaches combining specific technologies and substances. Further studies focusing on other kinds, optimal dosages and administration means should be carried on in the future.

Keywords: Statins, ACL Reconstruction, Tendon-bone Healing, Osteogenesis, Angiogenesis

1. Introduction

The anterior cruciate ligament (ACL), one of the basic components to stabilize the knee joint and provide normal knee kinematics, can be injured when participating in sports [1]. Under this injuring condition, ACL reconstruction is often recommended as a gold standard with grafts including autograft, allograft and synthetic graft [2]. Since ACL reconstruction is operated through fixing the graft with other specific materials like screws on the created bone tunnel, the tendon-bone interface requires a long healing period for reformation of the original four-layer microstructure, which consists of ligaments, non-mineralized fibrocartilage, mineralized cartilage and bone, to supply enough mechanical strength [3]. The tendon-bone healing is not only the main factor of the curative effect of reconstruction but it also affects the risk of secondary injuries with time to the surgery. In recent years, statins have been reported to possess pleiotropic effects that include improvement of endothelial effect, anti-inflammatory effect, and stimulation of angiogenesis [2]. Additionally, statins have an influence on bone metabolism which plays a part in promoting the tendon-bone healing after ACL reconstruction. This review systematically examines the potential of statins to enhance tendon-bone healing following ACL reconstruction based on literature from PubMed, Web of Science, and CNKI, by means of setting the sights on research directly operated on ACL animal models, other related animal models, combination use and mechanisms of usage. This review is

significant for elucidating the role of statins in enhancing tendon-bone healing following ACL surgery, as well as outlining future directions for preclinical research and clinical applications.

2. Methodology

The databases of Web of Science, CNKI, and PubMed were queried from 2013 to February 1, 2025. The retrieval is conducted using search terms: "statin", "ACL reconstruction", "anterior cruciate ligament", "tendon-bone healing", "tendon healing", "osteogenesis", "bone formation", and "chondrogenesis", along with relevant conjunctions such as "AND" and "OR". A total of over 500 papers were sourced from the aforementioned three databases, resulting in the acquisition of 17 papers, predominantly empirical studies, with a limited number of review articles contributing to the discussion and summary. This selection followed specific exclusion criteria: (1) irrelevance to the research topic; (2) content redundancy; (3) unavailability for various reasons; (4) publication in English or Chinese; (5) preference for the most recent publications. The acquired papers fell into two categories to be discussed based on whether the study was conducted directly on the ACL model or not. For papers that focused on the promotion of statins for tendon-bone healing after surgeries in other joints as well as on therapeutic mechanisms and administration routes, they could also provide strands of evidence that could contribute to recovery in post-ACL reconstruction conditions; thus, these articles were sorted into the non-ACL-model-based ones.

3. Researches directly based on ACL models

In recent years, researchers operated a few experiments directly on ACL models when they were fixing their eyes on the promoting ability of statins on tendon-bone healing. Zhang, P. et al. studied simvastatin (SIM) under local controlled-release administration in both vitro and vivo[5]. The targeted grafts of the surgery in their experiment are polyethylene terephthalate (PET) artificial ligaments, which were coated with rat-tail collagen containing low-dose SIM microsphere or only collagen. The obtained samples were recorded respectively as SIM/COL/PET group and COL/PET group. The contents of vitro experiments and corresponding results were listed in Table 1.

Table 1: Contents of vitro experiments and corresponding results

Contents of vitro experiments		Results
Cell viability and adhesion		The SIM/COL/PET group was more biocompatible than PET without coating to provide more space for the BMSCs to grow, thereby promoting cell proliferation and migration.
Osteogenic effect of SIM	Alkaline phosphatase (ALP) activity assays	The SIM/COL/PET group showed significantly higher degrees of BMSC mineralization compared with the PET group ($P<0.05$), which the higher content of ALP suggests that the mineralization capacity of cells is stronger.
	Mineralization assays: Alizarin red staining	The mineralized deposition of the SIM/COL/PET group was significantly more than the other two groups (both $P<0.05$).
	Real-time polymerase chain reaction analysis	Compared with the PET group, the SIM/COL/PET group increased the expression of growth factors vascular endothelial growth factor (VEGF) and bone morphogenetic protein-2 (BMP-2) as well as ligament repair markers, including osteocalcin (OC) and runt-related transcription factor 2 (RUNX2), indicating that the controlled-release SIM had an influence on enhancing osteogenesis and angiogenesis in BMSC cultures (OC: $P<0.01$; others: $P<0.05$).

While in vivo study, they used adult New Zealand White rabbits to operate ACL reconstruction and then assessed the histological and immunohistological conditions and conducted mechanical testing. The SIM/COL/PET group significantly showed a higher mean load of failure than the other two groups when pulling out from the bone tunnel (week 8: both $P < 0.01$) and a greater stiffness (week 8: both $P < 0.01$). During the healing period, at week 4, there was no significant difference in load of value ($P = 0.2031$) and stiffness ($P = 0.0181$) between the treatment groups. Meanwhile, the group with SIM reformed a microstructure containing tissue, bone and cartilage at tendon-bone interface, which was considered as a better stage of recovery than thick fibrous scar tissue developed in COL/PET and PET groups. This showed that the results of in vivo and in vitro experiments corresponded to further demonstrate that the use of SIM led to a higher level of vascularization.

Japanese researchers Oka et al. applied different approaches on rabbit models to figure out this topic [2]. In this study, simvastatin was conjugated with gelatin hydrogel, through the chemical crosslinking of gelatin with glutaraldehyde, and implanted into both bone tunnels in ipsilateral knee joints, which contained graft derived from hamstring tendon. While the right knee was for the simvastatin-treated group, the left knee inserted only gelatin hydrogel as a blank control. However, even though histological, micro-CT and biomechanical evaluations gave the results to prove that SIM treatment could accelerate the healing in the graft-to-bone interface during the early stage, which angiogenesis and osteogenesis improved, there were no significant differences in stiffness during the three recovery periods (all $P > 0.05$), which were 2, 4 and 8 weeks after surgery, and only at week 2 the average load of failure was significantly greater in specimens treated with SIM (week 2: $P < 0.05$; week 4, 8: $P > 0.05$). The researcher explained this phenomenon with that fibrovascular tissue healing happened promptly after the graft had contacted the bone, which was dominant to mechanical strength at the week 2 group, whereas immature cartilage formed during the latter periods could not greatly enhance the strength. This study also paid attention to the bone tunnel area during recovery since the suspensory fixation device adopted in the reconstruction was thought to often enlarge the bone tunnel area. According to the significantly declined area in the simvastatin-treated group (week 2: $P < 0.05$; week 4: $P < 0.01$; week 8: $P = 0.06 > 0.05$), it held a prediction that simvastatin-mediated bone infiltration could limit such expansion in the early stage.

Another team led by Lin et al. in China chose gelatin sponges as carriers to locally release SIM to graft, which was derived from flexor digitorum longus, in the bone tunnel [6]. There was more neovascularization and osteogenesis in the experimental knees compared with the knees on the other side, which were treated simply with gelatin sponges, as indicated by results from week 2 to week 8. They concluded from the results that simvastatin could promote the tendon-bone healing inside the bone tunnel after ACL reconstruction, while gelatin sponges could serve as low-cost carriers easy to operate.

4. Researches based on non-ACL models

One research study by Ni, Q. et al. in which simvastatin was also carried by a gelatin sponge for Achilles tendon-bone healing was vital as a supplement to the research in knee joints [7]. It complementally provided references in stiffness calculation, micro-CT scan and 3D image reconstruction which ulteriorly proved the promotion of simvastatin to tendon-bone healing biomechanically as well as enhancement in bone mineral density at the tendon-bone interface and reformation of bone structures near the bone tunnel in the Achilles model. It also conducted an vitro experiment that a co-culture of BMSCs and tendon-derived mesenchymal stem cells (TDMSCs) undergoing osteogenic and chondrogenic differentiation respectively with or without SIM treatment. The differentiation of chondroblast was promoted better in 7 days which the effect weakened gradually later, while facilitation of osteogenic differentiation was more significant along with the growing amount of ALP. In a different way, Dolkart, O. and other researchers assumed and proved

that atorvastatin promoted tendon healing after rotator cuff tearing via enhancing Cyclooxygenase2 (COX2) activity and stimulating autocrine/paracrine prostaglandin E2 (PGE2) signaling to promote proliferation, migration and adhesion of tenocyte [8].

A study around rosuvastatin (RSV) based on guided bone regeneration on rabbit calvariae was processed by Ozer, T. et al. They attempted to locally use rosuvastatin with a xenograft to promote bone formation in three-dimensional reconstruction [9]. During the process of healing, results of the RSV group at week 12 after surgery that distinctly higher bone height and density values of the new bone trabeculae as well as total bone volume revealed rosuvastatin could stimulate bone formation mightily if locally administered.

Bayyurt et al. came to the conclusion that atorvastatin was able to protect cartilage tissues in osteoarthritis model in rats [10]. It also showed evidence by electronic microscope that healthy chondrocytes of cartilage tissues in the study group contained appropriate matrix structures, including the regular Golgi complex and granulous endoplasmic reticulum. Several researches uncovered that statins adjusted osteoblast and osteoclast differentiation through mediating factors which were crucial to the process of new bone formation, including BMP-2, glucocorticoid, transforming growth factor-beta (TGF- β), ALP, type I collagen and collagenase-1. One of these studies had verified that statins had no effect on FGFR3 protein expression which used to be predicted as a cause of the relieving effect of statin treatment on abnormal fibroblast growth factor receptor 3 (FGFR3) -related chondrodysplasia [11].

The relationship between the systematic use of statins and the reduced risk of fractures has been inadequately supported by retrospective observational and case-control studies, likely due to the extensive first-pass effect of statins; thus, local administrations are deemed more valuable and effective [12]. In addition to microsphere, gelatin hydrogel, and gelatin sponge previously mentioned, injectable calcium phosphate foams (i-CPFs) facilitated sustained and prolonged release of pitavastatin; concurrently, the utilization of a titanium membrane (TM) layered between fluvastatin-loaded poly(lactic-co-glycolic) acid (PLGA) also promoted and enhanced new bone formation [13,14]. In a separate trial, SIM was administered alongside platelet-rich plasma (PRP) to address injuries at the Achilles tendon-bone interfaces in rats. The combination promoted chondrogenesis in vitro, and the simvastatin-PRP gel enhanced the healing process [15].

5. Discussion

Operated on rabbits, the three studies directly based on models of ACL reconstruction could be virtually considered to simulate what happened after simvastatin was used in humans' knee joints after the graft transplantation. Authentically, promotion of multi-layer structure formation and acceleration of healing in the graft-to-bone interface could be explicitly verified by two of the researches; nevertheless, research operated by Oka could only prove that the treatment of simvastatin could improve early tendon-bone healing histologically but it had less significant influences on stiffness, especially as recovery time went on. By contrast, in research which applied PET graft and collagen, no significant differences in stiffness between the SIM contained group and the COL/PET group were found while the stiffness grew to be significantly greater than the other 2 control groups after 8 weeks. No explanations or predictions were offered in the studies for these disparate results. Furthermore, they imposed further restrictions on the selection of animal models and detection methodologies. The species differences between New Zealand White rabbits and Japanese White rabbits may influence the observed result variations. Moreover, exact quantitative evaluations for determining the optimal dosage of simvastatin were not utilized, considering the combination of enhanced therapeutic efficacy and the absence of inflammatory response under certain administration methods. Moreover, it should be emphasized that the three aforementioned studies only shown that

simvastatins could enhance tendon-bone healing post-surgery, without considering the potential effects of other statins on this recovery process.

When it came to research not based on ACL models, they were valuable references. Although some of the researchers also had limitations in experiment design, such as dosage and sampling time, they showed relatively thorough evidence about how statins could facilitate the healing process and how to reach a better therapeutic effect. For the result that rosuvastatin stimulated bone trabeculae formation, it could enter in consideration when studying the process that indirect insertion changed into direct insertion. In Bayyurt's research, it stated the chondroprotective effect of atorvastatin and the healthy cellular structures in normal chondrocytes; however, they did not explore the relevance between the phenomenon and the statin-mediated protective effect. In addition, Kuwahara, M. et al. proved that fluvastatin induced endogenous BMP-2 to promote chondrogenic differentiation of adipose-derived mesenchymal stem cells (ADMSCs) suggesting a potential mechanism or therapy for statins in accelerating stem cell therapy, including tendon-derived stem cells (TDSCs), on tendon-bone healing after ACL reconstruction [3,16]. Furthermore, an article reviewed and offered an idea of a combination of β -caryophyllene (BCP) and statins considering that BCP could complement anti-inflammatory, antioxidant, antimicrobial and analgesic effects as well as improve osteoblast activity and inhibit osteoclastogenesis [17]. In this case, dosages of BCP and statins could be reduced to limit the resistance and cytotoxicity.

In future research, conducting surgeries on a single knee in animal models during post-ACL repair can more accurately replicate clinical procedures in patients. Meanwhile, larger scales and more humanoid experimental animals need to be used in the experiments. It can also conduct trials evaluating the efficacy of various technologies utilized in the delivery of statins. Identifying a method that minimizes bodily irritation while effectively meeting drug release criteria could enhance statin therapy for tendon-bone repair. Furthermore, the differences in therapeutic effects between simvastatin and other statins require investigation. Subsequently, the exploration of combination therapies using statins and other modalities should be undertaken to provide tailored options for diverse patient populations, ensuring an equilibrium among optimal therapeutic outcomes, minimal adverse effects, and cost-effectiveness.

6. Conclusion

Statins have shown the capability of enhancing tendon-bone healing both histologically and biomechanically in patients after ACL reconstruction through not only adjusting osteogenesis, but also stimulating angiogenesis. Besides, locally administered with or by microspheres, gelatin hydrogels, gelatin sponge, i-CPF, PRP or TM-layered PLGA, statins can accomplish the therapeutic goal better. In this review article, several limitations are identified, including paper resource constraints and no support from a real experiment. Further studies need to focus on more kinds of statins and compare the exact effects among the statin family. Optimal dosages and administration routes of statin therapy should be examined considering factors which can influence patients' physiological conditions, such as age, sex, serum lipid level and malignant diseases.

References

- [1] Qian, L., & Liang, Q. (2018). *Advances in the biological research of tendon-bone healing after anterior cruciate ligament reconstruction*. *Chinese Journal of Anatomy and Clinics*, 23(3), 272-276. doi:10.3760/cma.j.issn.2095-7041.2018.03.023
- [2] Oka, S., Matsumoto, T., Kubo, S., Matsushita, T., Sasaki, H., Nishizawa, Y., . . . Kuroda, R. (2013). *Local Administration of Low-Dose Simvastatin-Conjugated Gelatin Hydrogel for Tendon-Bone Healing in Anterior Cruciate Ligament Reconstruction*. *Tissue Engineering Part A*, 19(9-10), 1233-1243. doi:10.1089/ten.tea.2012.0325

- [3] Shen, S., Lin, Y., Sun, J., Liu, Y., Chen, Y., & Lu, J. (2024). A New Tissue Engineering Strategy to Promote Tendon-bone Healing: Regulation of Osteogenic and Chondrogenic Differentiation of Tendon-derived Stem Cells. *Orthop Surg*, 16(10), 2311-2325. doi:10.1111/os.14152
- [4] Chamani, S., Liberale, L., Mobasheri, L., Montecucco, F., Al-Rasadi, K., Jamialahmadi, T., & Sahebkar, A. (2021). The role of statins in the differentiation and function of bone cells. *European Journal of Clinical Investigation*, 51(7). doi:10.1111/eci.13534
- [5] Zhang, P., Han, F., Li, Y., Chen, J., Chen, T., Zhi, Y., . . . Zhao, P. (2016). Local delivery of controlled-release simvastatin to improve the biocompatibility of polyethylene terephthalate artificial ligaments for reconstruction of the anterior cruciate ligament. *Int J Nanomedicine*, 11, 465-478. doi:10.2147/ijn.S95032
- [6] Lin, J., Sun D., Zheng X., Yin D., Li Y., & Zhang L. (2015). Enhancement of tendon-bone healing by topical use of simvastatin after ACL reconstruction. *Journal of Shandong University (Health Sciences)*, 53(3), 27-31. doi:10.6040/j.issn.1671-7554.0.2014.887
- [7] Ni, Q., Zhu, J., Li, Z., Li, B., Wang, H., & Chen, L. (2023). Simvastatin promotes rat Achilles tendon-bone interface healing by promoting osteogenesis and chondrogenic differentiation of stem cells. *Cell and Tissue Research*, 391(2), 339-355. doi:10.1007/s00441-022-03714-w
- [8] Dolkart, O., Liron, T., Chechik, O., Somjen, D., Brosh, T., Maman, E., & Gabet, Y. (2014). Statins enhance rotator cuff healing by stimulating the COX2/PGE2/EP4 pathway: an in vivo and in vitro study. *Am J Sports Med*, 42(12), 2869-2876. doi:10.1177/0363546514545856
- [9] Özer, T., Aktas, A., Avag, C., Firat, A., Ocak, M., Kaymaz, F. F., & Çelik, H. H. (2021). Evaluation of the effects of locally applied rosuvastatin on bone formation in a three-dimensional reconstruction rabbit xenograft model. *Turkish Journal of Medical Sciences*, 51(6), 3115-3125. doi:10.3906/sag-2011-109
- [10] Bayyurt, S., Küçükalp, A., Bilgen, M. S., Bilgen, Ö., Çavusoglu, I., & Yalçinkaya, U. (2015). The chondroprotective effects of intraarticular application of statin in osteoarthritis An experimental study. *Indian Journal of Orthopaedics*, 49(6), 665-671. doi:10.4103/0019-5413.168751
- [11] Fafilek, B., Hampl, M., Ricankova, N., Vesela, I., Balek, L., Kunova Bosakova, M., . . . Krejci, P. (2017). Statins do not inhibit the FGFR signaling in chondrocytes. *Osteoarthritis Cartilage*, 25(9), 1522-1530. doi:10.1016/j.joca.2017.05.014
- [12] Henssler, L., Kerschbaum, M., Mukashevich, M. Z., Rupp, M., & Alt, V. (2021). Molecular enhancement of fracture healing-Is there a role for Bone Morphogenetic Protein-2, parathyroid hormone, statins, or sclerostin-antibodies? *Injury-International Journal of the Care of the Injured*, 52, S49-S57. doi:10.1016/j.injury.2021.04.068
- [13] Khurana, K., Guillem-Marti, J., Soldera, F., Mücklich, F., Canal, C., & Ginebra, M.-P. (2020). Injectable calcium phosphate foams for the delivery of Pitavastatin as osteogenic and angiogenic agent. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, 108(3), 760-770. doi:https://doi.org/10.1002/jbm.b.34430
- [14] Furuhashi, A., Rakhmatia, Y. D., Ayukawa, Y., & Koyano, K. (2022). Titanium membrane layered between fluvastatin-loaded poly (lactic-co-glycolic) acid for guided bone regeneration. *Regenerative Biomaterials*, 9. doi:10.1093/rb/rbac061
- [15] Zhang, Y., Yu, J., Zhang, J., & Hua, Y. (2019). Simvastatin With PRP Promotes Chondrogenesis of Bone Marrow Stem Cells In Vitro and Wounded Rat Achilles Tendon-Bone Interface Healing In Vivo. *Am J Sports Med*, 47(3), 729-739. doi:10.1177/0363546518819108
- [16] Kuwahara, M., Akasaki, Y., Goto, N., Kurakazu, I., Sueishi, T., Toya, M., . . . Nakashima, Y. (2022). Fluvastatin promotes chondrogenic differentiation of adipose-derived mesenchymal stem cells by inducing bone morphogenetic protein 2. *BMC Pharmacol Toxicol*, 23(1), 61. doi:10.1186/s40360-022-00600-7
- [17] Marom, H., Khan, M. A., Darvish, N., Tornetta Iii, P., Khoury, A., Weil, Y. A., . . . Ehrmann Barr, T. (2025). β -Caryophyllene and Statins in Bone Fracture Healing - A Narrative Review. *Orthop Res Rev*, 17, 31-42. doi:10.2147/orr.S506427