Review Article

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Histological Changes in Anterior Cruciate Ligament after Injury

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Abstract

The anterior cruciate ligament (ACL) injury is very common worldwide, frequently associated with sports trauma. Often, ACL tear necessitates reconstruction by replacing the entire ligament. Despite ACL reconstruction being performed widely, there is still lack in restoring the anatomical functions. Histological studies have demonstrated that the injured anterior cruciate ligament has numerous vessels and fibroblasts proliferation potential with continuous collagen turnover after 13–20 weeks of injury. Therefore, the ACL has healing potential and the remnants of injured ACL can be used for repair/reconstruction procedures. A better understanding of histological characteristics of injured ACL ligament will add further knowledge for finding new treatment techniques for ACL repair.

Keywords: Ruptured anterior cruciate ligament, Histology, Vascularity, Collagen, Fibroblasts.

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Introduction

The human anterior cruciate ligament is an important structure in the knee responsible for the anterior knee stability by limiting the movement of anterior tibial translation over the femur. The anterior cruciate ligament injury is a common injury worldwide, frequently associated with sports injury. It places a significant load on the health-care system in the form of surgical treatment and post-surgery rehabilitation due to its poor spontaneous healing.

Various theories were proposed to explain the poor healing of anterior cruciate ligament. Most important of these were its poor vascularity, insufficient fibroblast proliferation and disordered collagen bundles. Thereby, the healing capacity of the ligament was reduced and consequently the primary repair resulted in an unacceptably high failure rate. Hence, conventional reconstruction procedure entailed removal of the native ligament and replacement with hamstrings or patellar tendon graft. Despite, vast number of reconstruction surgeries available, there was considerable failure rate of surgeries, owing to biological failure of graft. Remnant preservation is one alternative choices for augmentation of the graft and prevention of graft failure. Novel techniques include usage of bio-scaffolds, sutures, and other materials for augmentation of the native ligament.

Conventional reconstruction surgery had higher rates of osteoarthritis and loss of proprioception. Studies have shown that remnant preservation technique had a better clinical outcome than the conventional surgery. Thus, the preservation of native ligament would prove to be of benefit. Better understanding of the healing potential of the remnant of anterior cruciate ligament is mandatory for remnant preservation strategy to be implemented on routine basis. Review of microstructural architecture in anterior cruciate ligament injury and healing is essential for understanding the neovascularization potential and proliferation capacity which play a key role in the healing of ligament tissue.

Subjects and Methods

Search Engine: PubMed and Google scholar.

Inclusion criteria: Articles included in this review were related to the parameters on blood vessels, fibroblasts, collagen fibres in anterior cruciate ligament tear. Articles in the English language, human and animal studies were included.

Exclusion criteria: Studies involving prevention strategies of anterior cruciate ligament injury, surgical techniques of repair and articles other than the English language were excluded.

Review : The present review described under the parameters of ligament healing includes changes in vascularity, fibroblasts, collagen changes and the relevant demographical parameters.

Changes in vascularity

Studies carried out in animals and humans explored the changes in vascularity. Mastrangelo et al. observed the blood vessel density over weeks among Yucatan minipigs. They grouped the subjects as skeletally immature, adolescent and adult types based on epiphysis. At 1^{st} week immature animals had higher blood vessel density significantly than the adults. With respect to the thickness of regenerated vessels, at 1-week thin venules and arterioles were seen. At 2^{nd} week multi-layered vessels were seen with red blood cells in the lumen. High density of blood vessels was seen in the peripheral epi-ligamentous region than in the central wound site. Vessels were randomly oriented at 1^{st} and 2^{nd} weeks. By 4^{th} week all three groups showed capillaries in the central wound site.[1]

Nayak et al. conducted a study on tibial remnant of ruptured anterior cruciate ligament and their correlation with the duration of injury. They found out that blood vessel density was maximum in group III at 13–20 weeks which coincides with proliferative phase, minimum in group V after 50 weeks which coincides with phase of reorganization when the pro-angiogenic factors were reduced.(Group I ($\leq$ 6 weeks; N = 16), Group II (7–12 weeks; N = 18), Group III (13–20 weeks; N = 7), Group IV (21–50 weeks; N = 12), Group V (> 50 weeks; N = 7).[2]

Sonnery-Cottet et al. found significant angiogenesis in 54% cases of anterior cruciate ligament tear.[3] Murray et al. studied the phases of ACL healing, they found out the dilated arterioles and venules with hyperplasia of smooth muscle cells.[4]

Trocan et al. used CD34 to assess the blood vessel density of the ruptured anterior cruciate ligament and compared with intact ligaments. CD34 positivity was seen in endothelium with high intensity and perivascular cells of synovium with granular and discontinuous pattern with moderate intensity and in fusiform cells located in the periphery of ligament close to synovium and in the fibrocartilage with discontinuous pattern with moderate intensity. They divided the samples into two groups, one with microlesions and the other without microlesions. The mean blood vessel density in ligaments with microlesions was found to be 43 per field (at X200), compared to 15 per field (at X200) in ligaments without injury.[5]

Newly formed vessels in synovium were seen close to epithelia in the area of epiligament and mostly surrounded by CD34 positive fibroblasts. Great differences were noted in size and shape of synovial blood vessels indicating active neovascularization in the ruptured anterior cruciate ligaments. New vessels inside the ligament were observed in 22.4% of samples were immature, which were capillaries, small sized and the lumen was narrow. In 11% samples, newly formed intermediate vessels were small, irregular and the lumen was not visible. In 33% samples, two types of vessels include immature and intermediate vessels were seen suggesting neovascularization.[5]

Changes in cellularity

Various authors in the past researched on the proliferation capacity of the injured anterior cruciate ligament. Mastrangelo et al. study on Yucatan minipigs among the three groups over a week found that the cellular density increased drastically over the 1^{st} week following the injury in all three groups. There was a significant difference in cell numbers between immature and adult groups in proximal ACL region at 2 weeks following injury in proximal and central ACL regions and between adolescent and adult groups in central ACL region. At 1^{st} week, fibroblasts were randomly oriented in all age groups. At 2^{nd} week, immature and adolescent groups presented with random orientation of fibroblasts whereas adult group showed regular orientation of fibroblasts. At 4 weeks, all groups showed regular arrangement of fibroblasts. At 1-week cells at all wound sites in all groups were ovoid and large significantly.[1]

At week 2, cell size reduced, shape of the cells was ovoid in immature group and fusiform in adolescent and adults. At 4 weeks, all age groups showed fusiform shaped cells. With respect to the size of nucleus, there was significant difference between 1^{st} week, 2^{nd} week and 4^{th} weeks in immature and adult models. Large ovoid fibroblasts denote the active fibroblasts. Higher proliferation does not guarantee higher synthetic activity and a better outcome. Immature and adolescent animals showed faster proliferation but the matrix is less uniformly distributed. Higher cell number is known to be a precursor of increased strength of healing ligament. High cell density in immature and adolescent animals denoted high proliferation and migration potential. Both proliferation and migration are found to be essential for a good wound healing.[1]

Neurath et al. found fibroblast proliferation started at 3 days and was maximum at 25–45 days then reduced later.[6] Nayak et al. observed fibroblast density to be least in group I, which was less than 6 weeks coinciding with epi-ligament reparative phase, significantly increased in 7–12 weeks signifying proliferative phase, reached maximum at 13–20 weeks, then reduced after 20 weeks signifying remodeling and maturation phase. Significant difference was found between group I and II, I and III, I and IV, II and V, III and V.[2] (Group I ($\leq$ 6 weeks; N = 16), Group II (7–12 weeks; N = 18), Group III (13–20 weeks; N = 7), Group IV (21–50 weeks; N = 12), Group V (> 50 weeks; N = 7).

Table 1: Reported methods with different histological parameters on ACL injury and healing

S. No	Authors	Year	Cases	Controls	Type of study (stains)	Parameters
1	Nayak et al.	2020	Patients with symptomatic ACL tear	-	H&E, IHC, TEM	Collagen fibril thickness, blood vessel density, fibroblast density
2	Everhart et al.	2017	Patients with ACL tear	Cadavers	H&E, Periodic aldehyde fuchsin, Masson's trichrome	Cellularity, collagen fibril organisation, vascularity
3	Trocan et al.	2016	Patients with ACL tear	Normal humans	H&E, Masson's trichrome, IHC	Blood vessel density, fibrocytes, stromal cells
4	Sonnery-Cottet et al.	2014	Patients with partial ACL tear	-	H&E, IHC	Cell density, blood vessel density, mechanoreceptors
5	Mastrangelo et al.	2010	Yucatan minipigs	-	H&E, IHC	Cell density, blood vessel density, nucleus shape and size, area of nucleus
6	Murray et al.	2000	Patients with ACL tear	Normal humans	H&E, IHC	Cell density, blood vessel density, nuclear morphology, alpha-smooth muscle actin positive cells
7	Neurath et al.	1994	Patients with complete ACL tear	Cadavers	TEM, IHC	Ultrastructure

H&E–Haematoxylin and eosin, IHC–Immunohistochemistry, TEM–Transmission electron microscope, ACL–anterior cruciate ligament.

Murray et al. found out that the in intact ACL, from proximal to distal the cell number density and blood vessel density reduced, sphericity of cell nuclei increased, alpha-smooth muscle actin positive cells increased. In injured ligament, after three weeks, there was no change in cell density in central area of ligament. Inflammatory cells were reduced, whereas fibroblast number increased. Epiligament showed increase in cell numbers. At 8 weeks, ligament remnant showed increase in cell numbers. Fibroblasts dominated the cell type. Highest cell density was seen at 16–20 weeks. Fibroblasts were disorganized. Many cells of synovial layers showed staining for alpha-smooth muscle actin. At 1 to 2 years, remodelling and maturation occurred. No connection was seen between the remnants. Fibroblasts were fusiform shaped, organized along longitudinal axis of ligament. Cell numbers reduced to usual numbers in an intact ligament. In medial collateral ligament healing of rabbits, it was found that fibroblast proliferation occurred after inflammatory phase, whereas epiligamentous repair phase in anterior cruciate ligament healing resulted in failure of healing.[4] They found contractile myofibroblasts based on anti-alpha-smooth muscle actin antibody reactive cells in the synovium during the repair phase around 8–12 weeks. Similar features as in other ligaments are fibroblast proliferation, neovascularization was also observed. Alpha-smooth muscle actin positive cells were thought to be responsible for the retraction of the torn ends of anterior cruciate ligament in humans. In healing process outside the joint, routinely fibrin clot forms, fibroblasts reach and replace the gap by collagen fibres. In the context of anterior cruciate ligament, fibrinolytic enzymes were demonstrated in the synovial fluid thereby inhibiting the above process, hence resulted in absence of tissue bridging, denoting the delayed healing process.[4]

Trocan et al. compared the ruptured anterior cruciate ligaments with the intact ligaments, 85% ruptured ligaments showed CD34 positive fibrocytes amidst the collagen fibrils whereas only 10% intact ligaments had CD34 positive fibrocytes. Scheffler et al. found hypo-cellularity in the beginning, followed by cellular proliferation from 4–6 weeks till 3 months, followed by remodelling for one year. Everhart et al. found increased cell density in acute tear of 4 weeks duration and chronic tear of 4 years duration, in contradiction with the

Table 2: Reported histological findings on ACL injury and healing

S. No.	Authors	Year	Blood vessel density	Fibroblast density	Nuclear morphology	Collagen
1	Nayak et al.	2020	Peaks at 13–20 weeks (6.28 vessels/mm^2) Least after 50 weeks (0.68 vessels/mm^2)	Peaks at 13–20 weeks (1095 cells/mm^2) Least below 6 weeks (471 cells/mm^2)	-	Collagen fibril thickness Peaks after 50 weeks (72 nm) Least at 7–12 weeks (43 nm)
2	Everhart et al.	2017	-	-	-	Continuous collagen turnover in acute and chronic tears
3	Trocan et al.	2016	43 per field (X200)in injured, 15 per field (X200)in intact 83% injured showed synovial angiogenesis, 20% intact showed synovial angiogenesis	94% injured ligaments showed stellate stromal cells, fibrocytes, 10% intact ligaments showed fibrocytes	-	-
4	Sonnery-Cottet et al.	2014	High blood vessel density in hyper-cellularity areas	54% cases had hyper-cellularity, 46% had hypo-cellularity	Ovoid nuclei in hyper-cellularity Fusiform nuclei in hypo-cellularity	-
5	Mastrange et al.	2010	Peaks at 2 weeks, Least at 4 weeks	Peaks at 4 weeks, Least at 1 week	Nuclear area peaks at 1 week, least at 4 weeks	Collagen content was most at 4 weeks, Orientation was disorganised at 1 week, parallel to axis at 4 weeks
6	Murray et al.	2000	Peak (13/mm) at 16–20 weeks Least (2/mm) at 52–104 weeks	Peak (2300/mm^2) at 16–20 weeks Least (500/mm^2) at 52–104 weeks	Fusiform nuclei in 1–2 years	Collagen fascicles were disorganised initially then parallelly aligned with time
7	Neurath et al.	1994	-	Peaks at 25–45 days	Dilated cisternae of RER, intracytoplasmic lipid droplets, numerous intermediate filaments, irregular ribosomal accumulations	Type III procollagen peak at 4–7 days Collagen fibrils were stellate in 3 days–6 weeks

theory of hostile synovium.[7]

Changes in Collagen

Different studies observed the collagen response to the anterior cruciate ligament injury. At 2 weeks after injury, Mastrangelo et al. found that collagen fibres in adult minipigs

showed randomly orientation, whereas no fibres were found in immature an adolescent group. At 4 weeks, trend towards parallel arrangement of fibers was seen in all age groups along the axis of ligament.[1] Robin et al. found collagen III appearance by 6 weeks. Nayak et al. found decrease in collagen III between 7 and 12 weeks, then increased after 12 weeks. After 20 weeks, collagen I increased. Collagen fibril thickness was maximum at 50 weeks after injury and minimum at 7–12 weeks. Nguyen et al. found increased collagen III between 30 and 64 weeks, which did not influence thickness of collagen.[2] Murray et al. observed at 1 to 2 years, collagen fascicles were aligned well along axis of ligament.[4]

Everhart et al. studied on the collagen cross-link content after the anterior cruciate ligament injury. They found the immature cross-linking to be quite low which suggested a high turnover of collagen in all samples irrespective of acute and chronic injuries and irrespective of contact and non-contact injuries. It demonstrated the healing did not stop at any duration of injury. There was a continuous remodelling phase going on in the torn ligaments, which showed a great healing potential in ruptured anterior cruciate ligament irrespective of the duration of injury, mode of injury and age of patient. This great potential of healing seemed to be curtailed by the lack of formation of blood clot suggesting it could repair on its own if a bridging scaffold was provided. This could open doors to usage of biologic scaffolds. A pattern towards higher probability of collagen cross-linking content was noted if operated less than 12 months from the time of injury. Histology showed high turnover of collagen fibrils in an acute tear of 4 weeks duration which was against the theory of synovium being hostile. Acute anterior cruciate ligament tear showed high variability in the orientation of collagen fibrils as against the normal ligament. Chronic tear showed even higher amount of disorganization of collagen fibril.[7]

Effects of duration of injury and other demographical parameters

Trends of angiogenesis and proliferation potential with timeline were explored. Nayak et al. observed no correlation between collagen fibril thickness and duration of injury. They found positive correlation between fibroblast density and duration of injury and a negative correlation between blood vessel density and duration of injury. Negative correlation was found between collagen fibril diameter and fibroblast density. Collagen fibril thickness was maximum at 50 weeks after injury and minimum at 7-12 weeks. Significant difference was found between fibril diameter of groups I and II, II and IV, II and V, III and V. (Group I ($\leq$ 6 weeks; N = 16), Group II (7–12 weeks; N = 18), Group III (13–20 weeks; N = 7), Group IV (21–50 weeks; N = 12), Group V (> 50 weeks; N = 7).Age, gender, side, injury to meniscus, adhesion to posterior cruciate ligament did not affect the fibroblast density, blood vessel density, collagen thickness.[2]

Murray et al. found that cell numbers in the ruptured anterior cruciate ligament were significantly affected by the duration of injury and the location of the biopsy segment in ACL. Cell numbers increased up to 16–20 weeks, then reduced from 20 weeks till 52 weeks. Morphology of the cell nuclei was affected by the location of biopsy segment in ACL but not by the duration of injury. Age and gender did not affect any findings. Proximal segment of the ligament had high nuclear ratio compared to the distal part. Blood vessel density was significantly affected by the duration of the injury and was maximum at 16–20 weeks of injury and location of biopsy segment in ACL did not influence percentage of the alpha-smooth muscle actin positive cells significantly.[4]

Optimal timing of surgery

Researchers suggested optimal timings for the surgery based on the observation from their studies. Locerbach et al. suggested 1-week delay for surgical reconstruction. Nayak et al. suggested 13–20 weeks as the most appropriate based on maximum fibroblast proliferation and neovascularization.[2] Murray et al. showed that the cells in human anterior cruciate ligament have potential to migrate onto a scaffold in the lab.

[Table 1] shows reported methods with different histological parameters on ACL injury and healing. [Table 2] shows reported histological findings on ACL injury and healing.

Conclusion

The anterior cruciate ligament was found to have greater healing potential, evident by the presence of sprouting of new blood vessels, appearance of numerous fibroblasts and increase in collagen content. Therefore, remnants of ACL have healing ability and can be used for ACL treatments. This review helps in understanding the histological process occurring in injured anterior cruciate ligament. It further throws light for choosing available treating options for injured ACL.

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