

Can the Body Slope of Interference Screw Affect Initial Stability of Reconstructed Anterior Cruciate Ligament?: In-vitro Investigation

Nazanin Daneshvarhashjin

KU Leuven

Mahmoud Chizari

University of Hertfordshire

SM Javad Mortazavi

Tehran University of Medical Sciences

Gholamreza Rouhi (✉ grouhi@aut.ac.ir)

Amirkabir University of Technology

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Abstract

Background

Superior biomechanical performance of tapered interference screws, in regard to reconstruction of anterior cruciate ligament (ACL), compared with non-tapered screws, has been reported in the literature. However, the effect of tapered interference screw's body slope on the initial stability of ACL is not studied yet. Thus, the main goal of this study was to investigate the effects of interference screw's body slope on the initial stability of the reconstructed ACL.

Methods

Based on the best screw-bone tunnel diameter ratios in non-tapered screws, two different tapered interference screw were designed and fabricated. The diameters of both screws were considered to be equal to bone tunnel diameter in one third of their length from screw tip, then they were gradually increased by 1mm, in the lower slope (LSTIS), and 2 mm, in the higher slope (HSTIS) screws. To simulate the ACL reconstruction, sixteen soft tissue grafts were fixed, using HSTIS and LSTIS, in synthetic bone blocks. Through applying sub-failure cyclic incremental tensile load, graft-bone-screw construct's stiffness and graft laxity in each cycle, and through applying subsequent step of loading graft to the failure, maximum load to failure, and graft's mode of failure were determined. Accordingly, performance of the fabricated interference screws were compared with each other.

Results

HSTIS, compared to LSTIS, provides a greater graft-bone-screw construct stiffness, and a lower graft laxity. Moreover, transverse rupture of graft fibers for LSTIS, and necking of graft in HSTIS group were the major types of grafts' failure.

Conclusion

HSTIS compared to LSTIS, by causing less damage in graft's fibers; reducing graft laxity; and increasing fixation stability, better replicates the intact ACL's behavior.

Background

Anterior cruciate ligament (ACL) reconstruction is one of the most common orthopedic surgical procedures [1], in which hamstring tendon auto-graft is frequently used to reconstruct the ruptured ACL [2]. For the case of hamstring graft fixation, interference screw has become a popular choice, and many biomechanical studies reported equivalent, or greater stability than other methods of fixation [3–5]. A wide variety of interference screw body geometries, for instance, cylindrical, tapered and hybrid were patented [6–11], which are designed to provide acceptable stability by creating squeezing pressure that hold tendon graft into contact with the bone tunnel [12]. The interference screw needs to provide

sufficient strength and stiffness, which are necessary for rehabilitation and daily activities, before biological fixation is fully occurred [13].

Despite all advancement made in regard to the ACL reconstruction, fixation of the graft in tibial bone tunnel, in the immediate postoperative period, due to poor bone mineral density, also because of the direction of applied load, which is mostly in the direction of bone tunnel, is still a serious challenge [14–16]. Ten to twenty five percent of patients still suffer from graft failure in the initial stage of rehabilitation [17], and in 9–22% of reconstructions, clinically important increase in anterior knee laxity was reported [18]. Moreover, the observed slippage of the graft might be attributed to the micro-motion between the graft and the interference screw within the bone tunnel under cyclic loading, which would eventually lead to loosening of the graft [19]. Graft irritation, and laceration caused by metal interference screws could be another reason of some clinical failures [19, 20]. Furthermore, even a well-functioning ACL was reported to be at the risk of traumatic rupture with a pooled rate of 5.8 %, at a minimum of 5-year follow-up [21].

Previous *in-vitro* biomechanical studies throughout applying cycling loading, as well as loading grafts to failure, investigated the effect of interference screw's length, diameter, material and different manufacturer designs on critical clinical outcomes, such as displacement and strength of the fixed graft, and its mode of failure [3, 22–28]. Investigation on the effect of bone tunnel-interference screw diameter ratio implies that use of a small diameter screw may cause graft slippage from bone tunnel, and larger screw diameter may damage graft [12]. Micucci et al. found that screw diameters ranging from 1 mm less- to 2 mm greater than bone tunnel diameter can provide satisfactory fixations [3]. It was also found that fixation of tapered screws in tapered bone tunnel provides greater resistance to interference failure, compared with non-tapered screw in cylindrical bone tunnel, when the clearness between screw bone tunnel was equal in two groups [29]. On the other hand, an important aspect of ACL reconstruction is to learn from, and mimic the intact insertion site of the ACL into tibial bone, which consists of four zones as follows: parallel fibers ligament; non-mineralized fibrocartilage; mineralized fibrocartilage; and bone [30]. At the insertion site, the gradient in material properties of the ACL allows effective load transfer, and thus minimizes stress concentration, and consequently reduces damage [30]. For this reason, in reconstruction procedure, by using a constant screw-bone tunnel ratio, the natural connection of the ACL into tibial bone cannot be followed. Considering the currently available literature, it seems that a biomechanical comparison between performances of tapered interference screws with different body slopes, considering acceptable range of screw-bone tunnel diameter ratios [24], is missing.

The main objective of this study was to investigate the effects of gradual increase of the diameter of tapered interference screws from equal diameter with the bone tunnel, known as a best screw-bone tunnel ratio, in one third of their length from screw tip where the engagement of screw and bone starts, to a 2 mm larger-, compared to a 1 mm larger than the diameter of bone tunnel, on the stability of the reconstructed ACL fixation. It was hypothesized that by fixation of the interference screw with the higher body slope, compared to the lower one, intact ACL's attachment to the bone tunnel can be better replicated, and thus it can provide a more stable graft fixation.

Materials And Methods

Design and fabrication of the HSTIS and LSTIS

Using a CNC TraubTx8 machine, prototypes of the two designed interference screws were made of CK45 steel, with a 30 mm length, a tapered body, and a flat head (Fig. 1). The screws had the same thread shape, with a 2.5 mm pitch, and 1.5 mm depth. The main difference between the designs of the two groups was the body slope of the screws. The diameter of both screws in one third of their length, from the tip of the screw, was equal to bone tunnel diameter, which was gradually increased to 8.5 mm, and 9.5 mm, i.e. 1 and 2 mm larger than bone tunnel diameter, and are respectively named lower slope tapered interference screw (LSTIS), and higher slope tapered interference screw (HSTIS) (Fig. 1). Therefore, in LSTIS group, the average of screw diameter in one third of its length from the tip (Fig. 1, region A) was larger, and in the rest of its length (Fig. 1, regions B and C) was smaller, compared to HSTIS group.

In-vitro Tests: Comparison between LSTIS and HSTIS responses

Sixteen fresh bovine extensor tendons were extracted from bovines, sacrificed at an industrial slaughterhouse. The extracted tendons were cleared of adherent muscle fibers and surrounding soft tissues, wrapped, and stored frozen at -20 °C in sealed plastic bags for 3 weeks, in order to be used as a soft-tissue graft [31]. On the day of testing, the tendons were thawed to room temperature (for 2–4 h), and all of them were kept moist with an 0.9% normal saline solution during the sample preparation and test procedure [30]. Open-ended bone tunnels with a diameter of 7.5 mm were also created in the rigid polyurethane foam blocks (Sawbones, Pacific Research Laboratories, Inc., WA), with a density of 320 kg/m³ to simulate dense cancellous bone of tibial bone tunnel [32]. After preparation of the soft tissue and bone tunnel samples, looped bovine extensor digitorum tendon strands with a total length of 80 mm, were sized to 7.5 mm circumferentially by use of an ACL graft–sizing block.

Grafts were inserted in prepared bone tunnels in two groups. Then, HSTIS and LSTIS were placed concentrically between graft strands in the direction of the bone tunnel. Three centimeter of the proximal end of the tendon strands were kept outside of the bone tunnel, and were secured in the custom-made rigs in Zwick/Roell (Amsler HCT 25–400), and bone blocks were also fixed with a custom-made fixture (Fig. 2a). Immediately after preparation of each graft-bone-interference screw samples, mechanical tests were carried out. By keeping graft strands perpendicular to the synthetic bone surface, the loading was applied parallel to the longitudinal axis of the tunnel (Fig. 2a), which is the worst-case scenario of load occurring in the human body[3] (Fig. 2b). Various loading steps applied to the grafts in this study, are as follows: (a) Pre-loading: sinusoidal tensile load, from 5 N to 20 N, with a frequency of 1 Hz, for 10 cycles; (b) Incremental sub-failure cyclic loading: tensile load, with a rate of 25 N/sec to a peak value of 100, 150, 200, 250 and 300 N, then unloading and leaving the graft to be in rest for 60 seconds after each loading cycle [23]; and (c) Loading graft to failure: tensile load with a rate of 20 mm/s up to failure (Fig. 3).

In each loading cycle of incremental sub-failure loading, the bone-screw-graft's stiffness; energy loss, i.e. the area of the hysteresis curve during loading and unloading; and graft laxity increase parameter were determined. The graft laxity increase parameter, i.e. the difference between position of the graft before loading in each cycle and its position after the resting time followed by loading and unloading in each cycle, was introduced by Scheffler et al.[29]. Moreover, in loading the graft to failure (step c), the bone-graft-screw's stiffness; maximum load to failure; total displacement of the graft; and mode of graft failure were all recorded. The total displacement of the graft was defined as the difference between initial position of the graft after pre-loading and its position at the failure point, along the longitudinal axis of the tunnel. Statistical analysis was conducted with GraphPad Prism software (GraphPad Software, Inc.), version 6. In all groups, nonparametric distribution of the data was found using Kolmogorow-Smirnow test [29]. Parameters of interest were statistically compared between the two groups using the Mann-Whitney U Wilcoxon rank-sum test [22].

Results

In the HSTIS group, by increasing the peak values of the load in incremental sub-failure cyclic loading to 200 N, 250 N and 300 N, one, two, and three specimens' fixation failed, respectively. On the other hand, fixation failure occurred for one, three and five specimens, in cycles with peak values of 150 N, 200 N and 250 N, respectively, in the LSTIS group. Moreover, due to technical errors, one of the grafts, out of 8 grafts, failed in HSTIS fixation procedure, so the number of total graft samples were seven and eight, for HSTIS and LSTIS groups, respectively.

An increase in the stiffness of the graft-bone-screw construct was observed in successive cyclic loading in each group (Fig. 4a). At the cycles with load peak values of 100 and 150N, the stiffness of the graft-bone-screw construct in HSTIS vs. LSTIS were significantly different as follows: 40.73 ± 10.7 N/mm vs. 27.82 ± 5.10 N/mm ($P < 0.05$); and 57.70 ± 8.04 N/mm vs. 42.71 ± 7.51 N/mm ($P < 0.01$), respectively (Fig. 4a). Moreover, graft laxity increase parameter for LSTIS group in all sub-failure cyclic load levels was greater than that of the HSTIS group (Fig. 4b). The graft laxity in LSTIS vs. HSTIS, showed a significant difference in cycles with load peak values of 100 and 150N, i.e. 1.29 ± 0.51 mm vs. 0.58 ± 0.38 mm ($P < 0.05$); and 2.49 ± 0.99 mm vs. 1.17 ± 0.56 mm ($P < 0.05$), respectively. Regarding energy loss, in all sub-failure cyclic load levels, more energy was dissipated in LSTIS group, compared to HSTIS group (Fig. 4c). A significantly greater amount of energy was dissipated in the LSTIS group, compared with the HSTIS group, at the cycles with load peak values of 100 and 150N, i.e. 86.46 ± 20.23 mJ vs. 49.12 ± 21.03 mJ ($P < 0.01$); and 151.00 ± 52.46 mJ vs. 93.04 ± 43.25 mJ ($P < 0.05$), respectively.

In loading grafts to failure (step c), even though no significant difference was observed between maximum loads at failure, and the stiffness of graft-bone-screw construct, between HSTIS and LSTIS groups (Table 1), but a noticeable difference between total graft displacements at failure was evident, between the two groups, i.e. 9.62 ± 1.42 mm for LSTIS group, compared with 7.31 ± 1.14 mm, for HSTIS group ($P < 0.1$) (Table 1). Moreover, the proximal site of fixation, near to loading site, i.e. region A, Fig. 1, was found to be the weakest section in all constructs. Different forms of graft failure were observed, i.e.

transverse detachment of grafts' fibers (Fig. 5a), necking of the middle section of the graft material (Fig. 5b), and necking of the graft in insertion area (Fig. 5c). Another noticeable point is related to the major types of grafts' failure in each group. Transverse cut of grafts' fibers for LSTIS, i.e. 62.5% of the samples of this group (5 out of 8), and necking of grafts in HSTIS, i.e. 71.4% of this group constructs (5 out of 7), were found to be major types of grafts' failures. Necking in the middle region of the graft was another type of the graft failure, which was seen just in one sample, out of 7 samples, in HSTIS group.

Table 1

Summary of results in loading the graft up to failure: Stiffness of graft-bone-screw construct, Maximum load at failure and Total graft displacement in both groups with their corresponding P values

Measured parameter	LSTIS	HSTIS	P value
Stiffness of graft-bone-screw construct (N/mm)	107.9 ± 6.4	123.4 ± 27.7	P = 0.152
Maximum load at failure (N)	292.8 ± 163.4	360.2 ± 155.6	P = 0.415
Total graft displacement (mm) *	9.6 ± 1.42	7.3 ± 1.14	P = 0.086
* It is defined as the difference between initial position of graft after preconditioning step of loading and its position at failure point, along bone tunnel direction.			

Discussion

In regard to the ACL reconstruction using interference screws, there are still some concerns, such as: risk of early graft fixation failure, slippage, and laceration, which need to be addressed. Considering that an intact ACL experiences a gradual increase in stiffness as it gets closer to the point of insertion into the bone [29], it was hypothesized here that by increasing the slope of the interference screw, and thus mimicking a natural ACL structure, the stability of fixation will increase. In order to check the validity of the hypothesis, two custom-made metallic interference screws were designed and fabricated, i.e. lower slope tapered interference screw (LSTIS), and higher slope tapered interference screw (HSTIS), and performance of the fabricated screws were compared through experimental tests on graft-bone-interference screw constructs. The diameters of both screws in one third of their length, from the tip of the screw, was equal to the bone tunnel diameter, and they were gradually increased to 8.5 mm (in LSTIS), and 9.5 mm (in HSTIS), i.e. 1 and 2 mm greater than bone tunnel diameter (Fig. 1). Thus, in LSTIS group, compared to HSTIS group, the average of screw diameter in one third of its length from tip (Fig. 1, region A) was larger, and in the rest of its length (Fig. 1, regions B and C) was smaller.

To compare the capability of HSTIS and LSTIS in improving the initial stability of reconstructed anterior cruciate ligament, stiffness of each graft-bone-screw construct was measured through applying a sub-failure incremental cyclic loading. Results of this work proved superiority of HSTIS group, in terms of the graft-bone-screw stiffness, compared to LSTIS group, especially in cyclic loading with the peak values of: 100, 150 N (Fig. 4a). Stiffness of intact femur-ACL-tibia complex of human cadaver knee, under approximately similar incremental cyclic loading protocol, was measured in Scheffler et al.' study [22].

Their reported mean stiffness of the samples' constructs at cycles with peak values of: 100, 200, and 300 N, were 43.8, 76.3, and 92.6 N/mm, respectively [22]. The mean stiffness values of the bone-graft-screw stiffness in HSTIS group of this work, for the same cycles as the ones used in Scheffler et al.' study, were 40.73, 68.99, and 102.24 N/mm. These values are closer to those of intact ACL in Scheffler et al.' study [22], compared with the LSTIS group, with mean stiffness of 27.82, 66.05 and 100.9 N/mm, respectively (Fig. 4a). Thus, it seems that fixation of the grafts with HSTIS better bio-mimicked intact ACL function, compared with LSTIS.

Graft laxity is another important concern associated with the ACL reconstruction when employing interference screws. The graft laxity can be caused by graft's fiber damages, and/or graft slippage from the bone tunnel, without including the elongation of the tendon graft itself. The graft laxities found in this work for both groups were initiated by loads that were well below the failure load (see Fig. 4b), and showed an increase when the peak load increased (see Fig. 4b). Moreover, mean graft laxity measured for graft fixed with HSTIS was less than that of LSTIS, especially at loading cycle with peak values of: 100 and 150 N, in which the difference between LSTIS and HSTIS laxities were significant ($p < 0.05$) (Fig. 4b). This observation regarding the graft laxity indicates that different body slopes of the screw will likely lead to different performance of the reconstructed ACL in early stage of rehabilitation. Nonetheless, results of this work showed that there is insignificance difference between graft laxities in two groups for the load greater than 150N. These insignificance differences may be due to the reduction of survived samples numbers in cycles with higher peak values, especially in LSTIS group, which can directly affect the statistical analysis's results.

By comparing the graft laxity parameter in HSTIS group (Fig. 4.b) with those reported in Scheffler et al.' study, using a similar protocol of loading, one may hold promise for superior behavior of the HSTIS to none-tapered metal interference screws [22]. In their study, the graft laxity was measured in the case of fixing Smith & Nephew RCI interference screw with a diameter of 7 mm and length of 25 mm in the bone tunnels, with diameters ranging from 8 to 9 mm [22]. The graft laxity, in load cycle with a peak value of 200 N, was reported to be 3.0 ± 3.8 mm [22], which is greater than the corresponding value for both HSTIS and LSTIS groups of current study, which were 1.16 ± 0.56 mm 2.49 ± 1.00 , respectively. Furthermore, in Miccuci et al.' study, graft laxities, in the case of fixation by screws with diameters equal to, 1 mm smaller, 1 mm and 2 mm greater than, the bone tunnel, were measured with a video analysis technique, and with photo-reflective markers, while the graft was experiencing a cyclic loading from 50 to 250 N at the frequency of 2 Hz, for a total of 1,500 cycles [3]. The least graft slippage in their study was reported to be 2.65 ± 2.38 mm, for the screw with a diameter equals to the bone tunnel [3], which is greater than the graft laxity measured for HSTIS, in cycle with a peak value of 250 N, i.e. 2.54 ± 1.02 mm. However, Miccuci et al.' results have been reported after applying 1500 cycles of loading, and the reported values for 100 cycle of loads in their study are less than the corresponding values for both HSTIS and LSTIS groups. As a result, preponderance of HSTIS to none-tapered bio-interface screws cannot be claimed in this study and it seems that manufacturing HSTIS with biodegradable materials can be deemed as a good option for improving its mechanical behavior.

Another cause of graft fixation failure in ACL reconstruction surgery through using interference screws, which can be observed clinically, is the graft laceration. In this study, in order to investigate the effect of body slope of the screws on grafts damage, graft mode of failure for each sample was recorded. It was found that the graft and screw engagement in region A was the weakest site in all constructs (Fig. 1). However, the mode of graft failure in HSTIS group was mostly necking of the grafts, and the samples did not fail due to screw threads cuts, which was mostly the mode of failure in LSTIS group (see Fig. 5a). In previous studies, type of graft failure was only determined in terms of graft slippage, deterioration of graft material or failure at the mid-substance of the graft [33, 34]. Thus, due to paucity of the data in current literature on the subject of grafts' fibers damages, comparison with previous work is not possible here.

Based on the evidence provided in terms of lower stiffness, greater graft's fibers damages, higher graft laxity and displacement in LSTIS group, compared with HSTIS group (Table 1, Figs. 4 and 5), it can be speculated that, in the ACL reconstruction surgery, through using a tapered interference screw, the risk of early graft fixation failure can be minimized through controlling the pressure and contact area of the screw and graft, by means of precisely determined body slope for the screw. It seems that in region B and C (Fig. 1), smaller average diameter of screw and lower body slope of LSTIS causes less friction, compared to HSTIS, which consequently lead to a greater displacement of the graft in the former group. Subsequently, the slippage of the graft in regions B and C will be transmitted to region A, near to the loading exertion point, due to the direction of the applied load, pulling the graft outside of the bone tunnel (Fig. 2). Finally, it can be deemed that this transmitted graft to region A, in LSTIS group, due to a larger mean diameter of screw (Fig. 1), compared to HSTIS, will be exposed to higher average contact pressure, which leads to a transverse cut of the graft's fibers (see Fig. 5). On the other hand, higher body slope of HSTIS group, in conjunction with a smaller mean diameter of the HSTIS in region A, compared to LSTIS, prevents transverse cut of the graft at the most vulnerable regions of the fixation (Fig. 5). Therefore, it can be suggested that in regions B and C, major slippage of the graft takes place, which could be transferred to hazardous region A that can cause further damages on graft fibers, thus an adequate contact pressure must be applied in region B and C, while high contact pressures should be avoided in region A.

The following points should be taken into consideration while one is trying to interpret results of this work. First, *in-vitro* tests' results can give us information about the initial stability, but they are unable to evaluate mechanical behavior of the bone-graft-interference screw construct after graft healing and remodeling processes, which can alter graft tissue's mechanical properties [35]. Secondly, stress distribution within the graft and on bone tunnel can have influence on bone tunnel widening during the healing and remodeling processes, and consequently can affect graft fixation stability, which was not taken into account in this study. Thirdly, it should be noted that extensor-digitorum of bovine [36, 37], instead of human hamstring tendon, was used in this investigation. Lastly, synthetic bone, similar to a dense cancellous bone, was used here, in order to avoid cadaver's wide range variation in BMDs, as well as non-homogeneity of real bone, and thus make the comparison between the LSTIS and HSTIS more logical.

Conclusions

In the ACL reconstruction surgery, by using the bio-mimicked tapered interference screw, the risk of early graft fixation failure can be reduced through controlling the contact area of screw and graft, and thus by adjusting pressure distribution at the screw-graft and graft-bone interfaces, by means of screw body slope, and consequently by adjusting the screw-bone tunnel diameters ratio gradient along the interference screw. Based on this study, it can be concluded that in area near the load exertion site, region A (Fig. 1), engagement of screw and graft can cause graft damages (Fig. 5), and thus high contact pressure should be avoided in that region. Moreover, major slippage of graft occurs in regions B and C (Fig. 1), which might migrate to the critical region A, and cause further damages, thus a proper graft fitting must be maintained in regions B and C. By increasing the diameter of the interference screw linearly, similar to the custom-made HSTIS of this study, with a greater body slope than that of LSTIS, through reducing the diameter of the screw in region A, and increasing the diameter in region B, a greater screw-bone tunnel diameter ratio gradient along the screw can be gained, and thus it can better bio-mimic the intact ACL attachment behavior. Since just two most probable efficient body slopes were studied here, in order to discover new aspects of the effects of body slope of the interference screw and answering the key question of “what is the best slope for an interference screw that can result in the most favorite outcome of ACL reconstruction surgery?” further attention and investigation needs to be made.

Abbreviations

ACL: Anterior cruciate ligament

LSTIS: Lower slope tapered interference screw

HSTIS: Higher slope tapered interference screw

Declarations

Ethics approval and consent to participate

All methods and experimental protocols were approved by Amirkabir and Sharif university of technology.

Availability of data and materials

The datasets used during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Not applicable.

Authors' contributions

Nazanin Daneshvarhashjin: Study design, data collection, interpretation of data, statistical analysis, writing the first draft of the manuscript and revision of the manuscript

Mahmoud Chizari: Study design, interpretation of data and revision of the manuscript

SM Javad Mortazavi: Study design and revision of the manuscript.

Gholamreza Rouhi: Study design, interpretation of data, substantively revision of the study.

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Figures

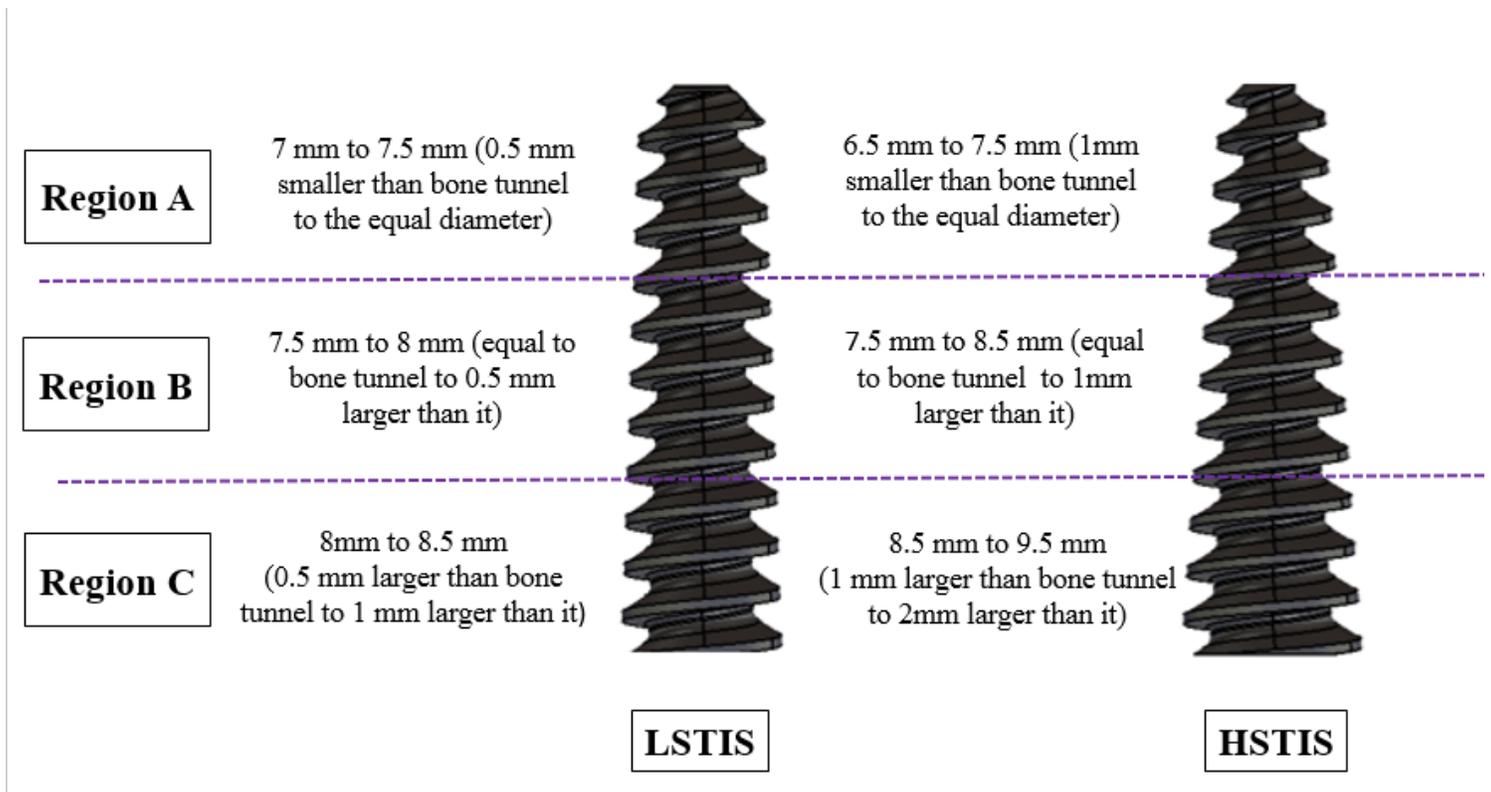


Figure 1

Changes in diameters of two tapered interference screws: (Left) Lower slope tapered screw (LSTIS), and (Right) Higher slope tapered screw (HSTIS). Through regions B and C, the diameters of LSTIS and HSTIS were increased from 7.5 mm to 8.5, and from 7.5 to 9.5 mm, i.e. 1 and 2 mm greater than bone tunnel, respectively.

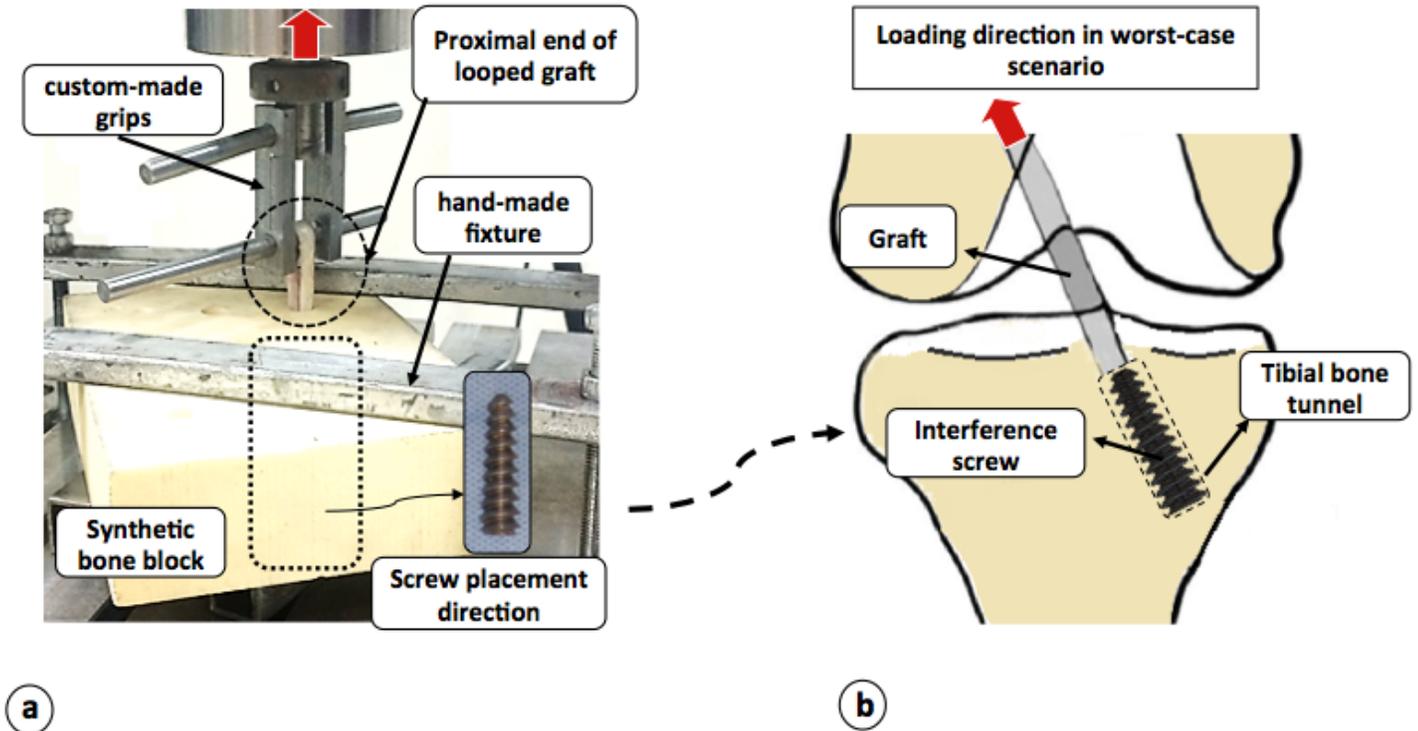


Figure 2

Experimental test set-up: (a) A looped graft captured in a custom-made rig, and fixed with an interference screw within a bone block, secured with a hand-made fixture, in the mechanical testing set-up; and (b) Position of tibial bone tunnel, direction of interference screw, and loading direction in the worst-case scenario in human body.

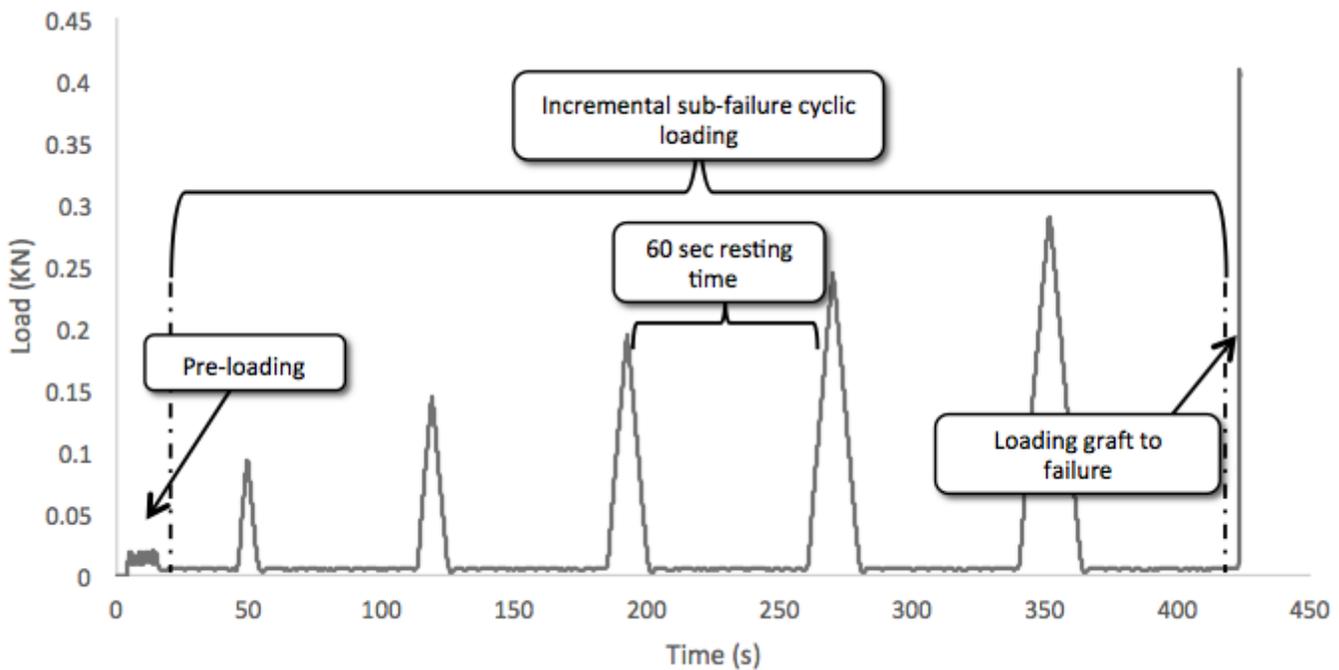


Figure 3

Loading steps in the current study: Pre-loading: sinusoidal tensile load changed from 5 to 20 N, with a frequency of 1 Hz; Incremental sub-failure cyclic loading: tensile load, with a rate of 25 N/sec to a peak value of 100, 150, 200, 250 and 300 N, then unloading and leaving the graft to be in rest for 60 seconds after each loading cycle; and finally loading graft to failure with a rate of 20 mm/s.

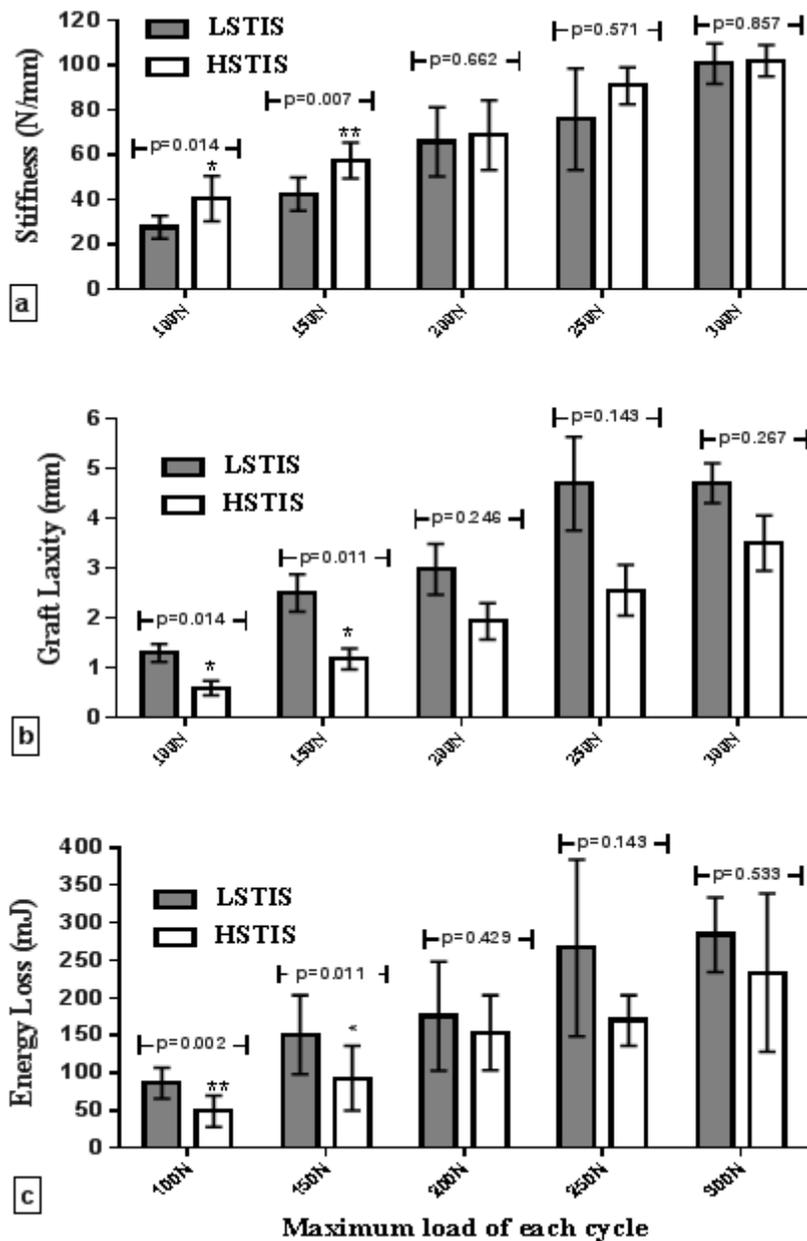


Figure 4

In-vitro tests results in different tensile sub-failure cycles: (a) Stiffness of the graft-bone-screw construct (N/mm); (b) Graft laxity parameter (mm); and (c) Energy loss (mJ) of low slope tapered interference screw (LSTIS) and high slope tapered interference screw (HSTIS) in different tensile sub-failure cycles, with a peak load value of 100, 150, 200, 250, and 300 N.

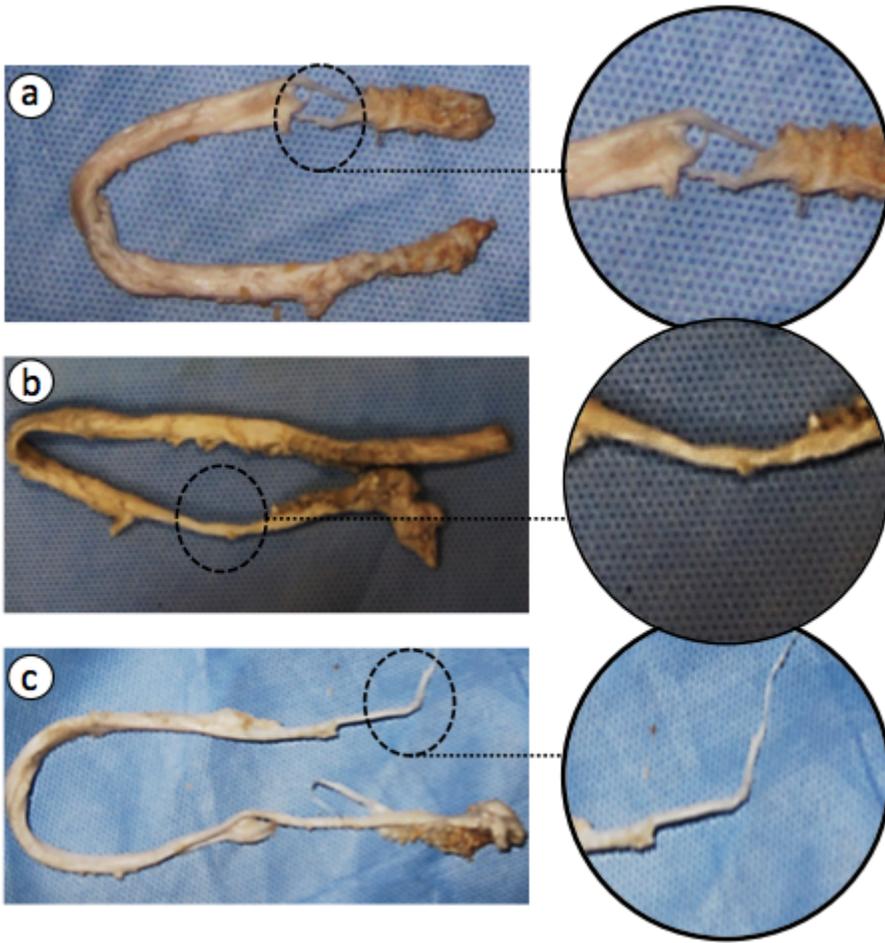


Figure 5

Three modes of the graft failure observed in this study: (a) Transverse rupture of graft's fibers near to loading site (region A, Fig. 1); (b) Necking of the middle section of the graft material; and (c) Necking of the graft in region A (Fig. 1).