How preconditioning and pretensioning of grafts used in Anterior Cruciate Ligaments surgical reconstruction are influenced by their mechanical time-dependent characteristics: Can we optimize their initial loading state?

by

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Structured Abstract

Purpose: Consensus about a pre-implant preparation protocol adaptable to any graft used in Anterior Cruciate Ligament (ACL) reconstruction is still lacking. In fact, there is not agreement on reliable metrics that consider both specific graft dimensional characteristics, such as its diameter, and the inherent properties of its constitutive material, i.e. ligaments or tendons. Aim of the present study was to investigate and propose *the applied engineering stress* as a possible metrics.

Methods: Preconditioning and pretensioning protocol involved groups of grafts with different section (10 or 32 mm^2) and materials (i.e. human patellar and hamstring tendons, and synthetic grafts). A 140 N load was applied to the grafts and maintained for 100 s. Initial stress and following stress-relaxation (a mechanical characteristic that can be related to knee laxity) were specifically analysed.

Findings: Initial stress, ranging between 4 and 12 MPa, was affected primarily by the graft cross-section area and secondarily by the choice of the graft material. In terms of loss of the initial stress, stress-relaxation behaviour varied instead on a narrower range, namely 13-17%.

Interpretation: Engineering stress can be identified as the correct metrics to optimize the initial state of each graft to avoid excessive stiffness, laxity or fatigue rupture phenomena.

Keywords:

Anterior cruciate ligament; graft; preconditioning; pretensioning; stress relaxation

Introduction

Anterior Cruciate Ligament (ACL) is one of the major ligaments within the human knee; It plays a critical role in joint stability since it avoids lateral dislocations of the femur with respect to tibia. Injuries of ACL are very common due to its prominent role and alteration of knee biomechanics as well as early development of osteoarthritis (OA) are some of the consequences of ACL damage [40]. These considerations lead clinicians toward ACL surgery reconstructions, in the last decade [32] that however– despite reporting promising short- to mid-term functional performance [39] – are not fully able to prevent premature knee OA [15] as well as to a return of complete physical activities [34].

The paradigm at the basis of ACL reconstruction implies the use of grafts to mimic the functional behaviour of the native ligament. The most common approaches primarily rely on autologous grafts - including patellar tendon and hamstring tendon -, biological allografts, xenografts and - mostly in augmentation solutions - bioengineered synthetic grafts [12][44] . However, besides the choice and execution of the surgical technique, there are many aspects that may influence the success of ACL reconstruction, among them: the typology of graft selection and the preparation of the implant. Inappropriate graft properties remain indeed a main issue that limits the long-term success of ACL reconstructions [17]. This aspect is well-known in clinical practice, where excessive laxity can be found in reconstructed knees as the effect of the time-adaptation of the used grafts [9][10]. In particular, the impact of viscoelastic characteristics on graft tensioning is critical [31][37]. In this perspective, preconditioning and pretensioning of the graft aim precisely to eliminate tension and stiffness decay after final fixation, but a shared and validated preconditioning/pretensioning protocol is still lacking [28]. Some recent clinical protocols have been proposed by Zheng et al. [43], that, however is difficult to generalize to different graft diameter from those used by the authors. Indeed, the effect of the initial stress on the hereditariness of different tendons of the human knee is still missing in scientific literature and only limited information about mammalian tendons may be found [31, 40]. Recently the effects of the initial elongations on the relaxation of some kind of fibrous tissue has been studied in stochastic context to assess a numerical predictive model for the mechanical features of the fibrous tissues [3].

In order to gain a robust and shared consensus about time-dependent behaviour of grafts used in surgical reconstructions, as first, the correct definitions should be introduced and shared. In this case, despite *tension/stress* appears as the fundamental terms – and in fact we talk about *pretensioning* [43] and *stress relaxation* [28] –, current *preconditioning* protocols are carried out in terms of applied *force*, while *stress* depends on the graft section area, i.e. graft geometrical characteristics.

The first assumption of this study is that – focusing on *stress* – a preconditioning protocol can be optimized with respect to the graft section, i.e. diameter, since geometrical factors affect, significatively, the results of the protocol [3]. The second assumption is that the protocol tuning on the graft diameter may be not sufficient to obtain an optimal outcome, because the grafts involves different tissue with different mechanical behaviour, although of the same size.

In this experimental study these assumptions are investigated to provide the effects of an "in vitro" mechanical test, mimicking a simple preconditioning/pretensioning protocol, and apply it to several graft samples used in ACL reconstruction. Final aim of this work is to identify reliable metrics able to support the optimal tuning of the initial loading conditions for any graft chosen in the treatment of ACL injury.

Materials and methods

Specimen selection and preparation

The experimental campaign involved two different types of human tissues, namely patellar (P) and hamstring (H) tendons, and a commercial synthetic graft (S). This choice was justified by the fact that these tissues are commonly used in ACL reconstruction [18]. They were collected from a Tissue Bank (*Science Care*, USA). The study was approved by the local Ethics Committee (protocol “TISS-KNEE” 8425). Tendons were stored at -80 °C and thawed in a 37 °C water bath for 15 min prior to testing [16] and then prepared by an experienced orthopaedic surgeon. After preparation, each specimen was cleaned and cut at the same length before clamping (Fig. 1). Four different groups were prepared:

- “H 32 mm^2”, an H group with section 32±11 mm^2, with pretensioning load of 140±18 N that corresponds to 4 MPa stress, equivalent to the pretensioning load of 160 N used in [43] for 7 mm diameter grafts and indicated as good for stiffness and strength.
- “H 10 mm^2”, an H group with section 10±11 mm^2, with pretensioning load of 140 N was applied.
- “P 32 mm^2”, a P group with section 32±1 mm^2, with a pretensioning load of 140 N was applied.
- “S 10 mm^2”, an S group as furnished by the manufacturer with section 10±1 mm^2, with pretensioning load of 140 N was applied.

Each group was composed of six samples, as indicated in [26].

Testing Procedure

The aim of this study is the definition of an “optimal” procedure to tune the initial loading conditions of grafts. The experimental set-up used to this aim has involved the use of a single-axis electroforce dynamic system (Bose 3330, TA instruments) to perform uniaxial tensile tests. Sample principal fibres were qualitatively aligned along the machine loading axis (Fig. 1.d). Clamping was obtained with the aid of milled grips, involving sandpaper for the synthetic grafts and a surgical basting suture at the end of hamstrings grafts (Fig. 1.a) [35]. In order to obtain reliable results, a specific experimental protocol consisting in four phases consecutively applied on the same sample was developed (Fig. 2):

1. *Preconditioning*: specimens were preconditioned by harmonic load between 20 and 100 N, for twenty cycles at 0.25 Hz, thus to remove any crimping in the tendon fibrils caused by prolonged storage in a fixed position [23].
2. *Recovery*: a 20 N load was maintained for 15 min to exhaust sample elongation. After recovery, the initial length of the specimen as well as its initial width and thickness were measured along three sections of mid-substance by using a standard digital caliper; measurements were repeated three times by two different operators and values were then averaged to calculate strain and engineering stress [25].
3. *Pretensioning*: a load was applied to induce pretension on the graft, considering a linear ramp with a loading rate of 315 N/s [13].
4. *Stress relaxation*: sample position reached in pretensioning was maintained for 100 s to mimic the behaviour of the grafts being implanted into the knee joint.

During the whole experimental test, specimens were continuously moistened with saline solution [22]. Stress relaxation lasted 100 s, that is an observation time chosen for accurate estimation of the phenomenon through a power-law model [33].

Data and Statistical Analysis

Mechanical characterization was obtained in terms of the engineering stress σ(t), obtained from the experimental data as $\sigma(t) = \frac{F(t)}{A_0}$, where $F(t)$ is the force measured during the uniaxial test and A_0 is the average cross-sectional area measured at the end of the recovery phase.

We denoted $\sigma(\underline{t})$ as the stress at the end of loading ramp ($t = \underline{t}$) and therefore modelled the stress relaxation as:

$$\frac{\sigma(t)}{\sigma(\underline{t})} = A t^{-\beta} \tag{1}$$

where $0 \leq \beta \leq 1$ is a decaying rate, expressing the speed of relaxation, whereas A is a coefficient of proportionality.

A specific comparison was carried out between the “H 32 mm^2” and “H 10 mm^2” groups to highlight the influence of graft diameter. Further, two more comparisons interested the “H 32 mm^2” and “P 32 mm^2” groups, and the “H 10 mm^2” and “S 10 mm^2”, respectively, to highlight the influence of graft materials.

Comparisons were realized in terms of pretension $\sigma(\underline{t})$, relaxation rate, β, and loading loss at 100 s, $\frac{\sigma(100) - \sigma(\underline{t})}{\sigma(100)}$, and were performed by using *Wilcoxon-Ranksum test* with a level of statistical significance *p-value* = 0.05.

Results

The experimental data from the mechanical tests reporting the normalized stress with superimposed power-law fitting, are reported in Fig. 3.

The results about the pretension level $\sigma(\underline{t})$ are collected in Fig. 4. The observation of the data shows that with a three times larger section A_0, the “H 32 mm^2” the group presented about a three times lower $\sigma(\underline{t})$ respect to the “H 10 mm^2” set; difference was statistically significant (*p-value* 0.0248). No difference was present between different materials with same section.

The relaxation rates β are collected in Fig.5. It can be observed that samples with larger section, in particular the “H 32 mm^2” set, shows faster relaxation if no statistical difference is present.

Loading loss, $\frac{\sigma(100)-\sigma(\underline{t})}{\sigma(100)}$ (Fig. 6), is strictly connected with β, as expected, since the faster is the relaxation, the higher is the tension decay in a given time interval. Thus, the “H 32 mm^2” showed the highest decay, but no statistical difference is present.

Discussion

The first assumption about the influence of the *stress* in the role of the ACL graft cross-section area (i.e. diameter), was specifically demonstrated by comparing grafts of the same typology, i.e. hamstring tendons, including different section areas.

The second assumption, about the importance of the graft tissue, arise from the need to tune the preconditioning/pretensioning protocol for each graft. However, the protocol did not show any significantly different effect depending on the graft typology at least for the selected test conditions and analysis.

The proposed preconditioning and based-on-*stress* pretensioning protocol shows comparable values of graft loss of tension after fixation, with respect to literature [36][14]. These findings further underlines the strong effect of pretensioning and hence the need to define a proper protocol in clinical applications.

The influence of the graft cross-section has been observed primarily on pretension and also, with less extend on stress relaxation. In particular for H, the test yields an inverse relationship between graft area and pretensioning and a direct relationship between graft size and relaxation. The results obtained for P are in agreement with literature [1].

The pretensioning load influences the mechanics of the grafts to a large extend [43]. The *Stress*, defined as force on section area, appears as the correct metric to take into account both the contributes, with the aim to identify an optimal approach for graft selection and preparation. In fact, the inherent definition of stress could help comparing and exploiting the results obtained in different studies. For instance, indications about a "dangerous" pretension – i.e. an excessive applied force able to introduce alterations in structure –, seem to be different if we consider a load of 340 N for a hamstring graft [43] and a load of 500 N for a hamstring-polyethylene hybrid transplant [19]. Nevertheless, focusing on cross-section area, we found that the first study used a graft diameter of about 7 mm, whereas the second of about 9 mm, thus both leading to a common indication in terms of *stress* (for both about 8 MPa).

Indeed, stress is fundamental in interpreting structural-mechanical relationship [5][7][6][8]. The previously reported stress value of 8 MPa represents an important basis also to interpret our results. While Hingorani et al. [27] and Vena et al. [41] showed that a higher pretension load led to a higher level of relaxation, the behaviour identified in this work appeared to be quite different, presenting lower level of relaxation when higher stress was applied; anyhow in [27] only pre-damage strains were used and it was speculated that a separate behaviour may be identified at higher levels of induced stress. This could be indeed our case, since the higher stress values are all above the critical value of 8 MPa, thus micro-damages could have impaired a full reorganization of the tissues and therefore the overall relaxation behaviour.

In the analysed testing conditions, the influence of different graft materials was not that evident. Despite few differences due to the proposed testing protocols, consistency with literature was specifically identified in tension level and rate of relaxation for both P and H samples [43][29][1][24][13]. Unfortunately, no data for comparison were found in literature concerning synthetic graft, with particular attention to the specific polyethylene terephtalate material.

Despite this study did not find different macroscopic behaviours related to the several grafts tested, their specificity should not be ignored. Micro-structural differences were in fact reported between hamstring and patellar tendons [20][21]. Although these grafts are of the same typology of tissue, they were harvested from different knee anatomical locations, definitely with a specific different function. For this reason, under mechanical test conditions different from these observed in this study, some significant differences were highlighted [42]. For the analysed synthetic solution, differences with respect to hamstring were not attended in general, as hamstring graft represents a "gold standard", thanks to a broad range of available analyses [13][30][11][19]. Nevertheless, new materials are under development to obtain optimal ACL graft properties [2], therefore focusing on *stress* - here proposed as one possible tuning metrics - can be useful to identify the optimal graft diameter.

This study presented several limitations and that may be improved in the close future. Concerning testing, despite the use of several solutions to avoid slippage of the soft tissues, further experiments should necessarily consider the clamping performed on bone insertions or the use of special gripping techniques [38][4]. The obtained results show that the major limitation pertains to the small sample size and testing conditions, that did not allow us to completely generalize relationships between graft diameters and materials. Nevertheless, this study provided an important basis that can be used as a preliminary approach to optimize in particular the preconditioning/pretensioning protocol and, more in general, the choice of graft in the treatment of ACL injury.

Conclusions

A preconditioning and based-on-*stress* pretensioning protocol was proposed to reduce stress decay of ACL grafts after fixation within the knee joint. *Stress*, defined as force on cross-section area, resulted a useful metric to optimize the surgical protocol and can be used to reach a consensus in the preconditioning/pretensioning approach depending on graft choice. Furthermore, the presented findings supported the necessity to analyse the static and time-dependent mechanical behaviour of standard and innovative graft solutions, to increase the basic knowledge about them with the perspective of obtaining optimal clinical and functional outcomes.

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Figure Captions:

Figure 1. Preparation for testing of: hamstring tendon sample H (a), patellar tendon samples P (b), synthetic ligament S (c). A P sample clamped in the traction machine (d).

Figure 2. Scheme of the testing protocol, where F is the vertical force read by the load cell.

Figure 3. Stress relaxation dimensionless mean curves (markers) for patellar P, hamstring H and synthetic S samples with section area 32 or 10 mm^2, and relative power fitting superimposed (straight lines). σ(t) stands for tension at time t, σ(t̲) is the tension at the beginning of the relaxation (pretension).

Figure 4. Pretension $\sigma(\underline{t})$ for patellar P, hamstring H and synthetic S samples with section area 32 or 10 mm^2.

Figure 5. Relaxation rate β for patellar P, hamstring H and synthetic S samples with section area 32 or 10 mm^2.

Figure 6. Loading loss, $\frac{\sigma(100)-\sigma(\underline{t})}{\sigma(100)}$ for patellar P, hamstring H and synthetic S samples with section area 32 or 10 mm^2.

Figures:

Fig. 1 a-d

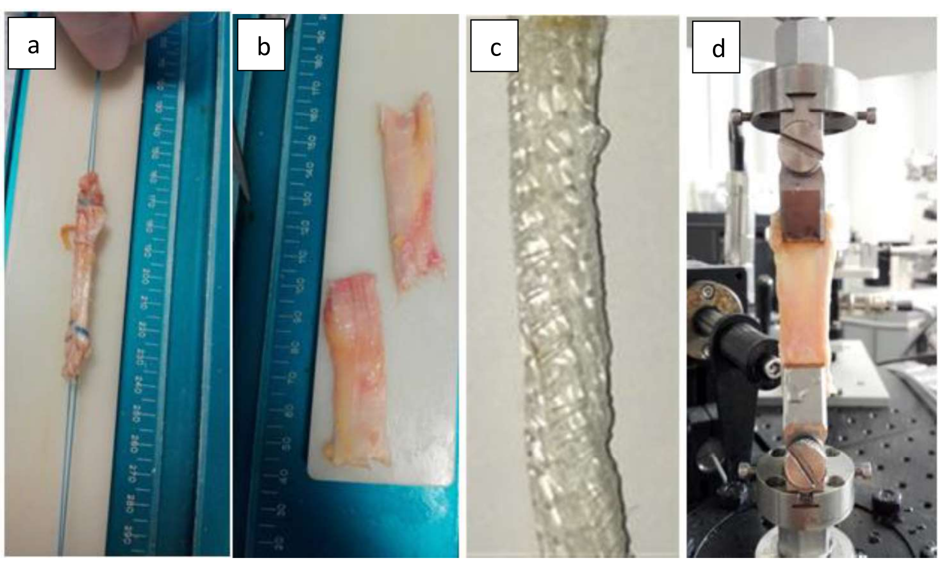


Fig. 2

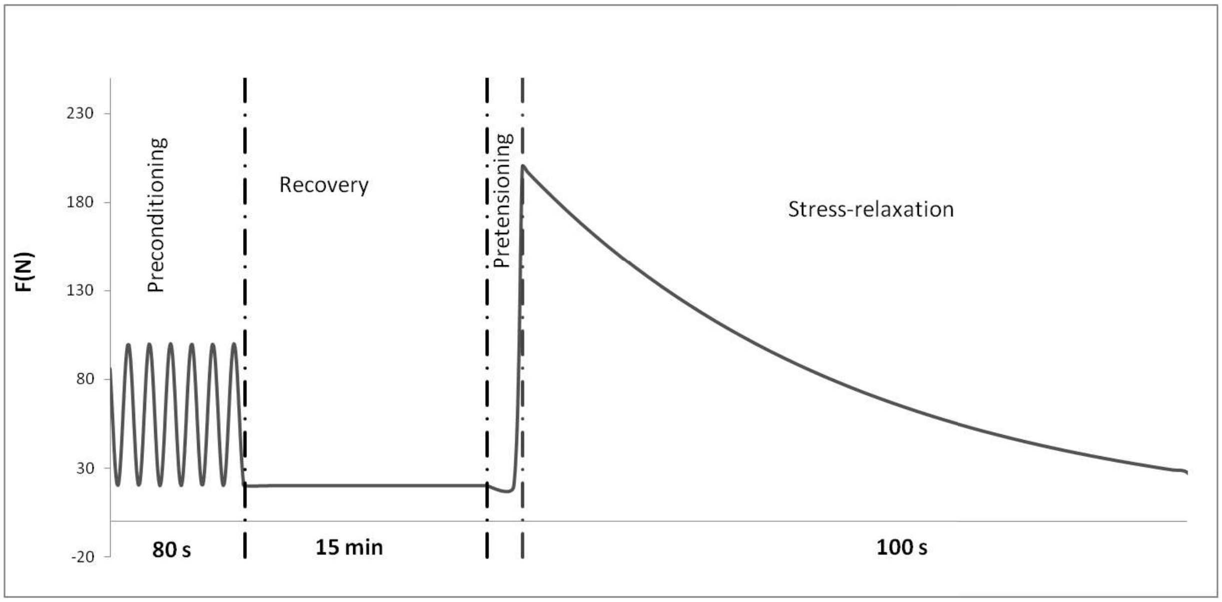

F(N)
230
180
130
80
30
-20
Preconditioning
Recovery
Pretensioning
Stress-relaxation
80 s
15 min
100 s

Fig. 3

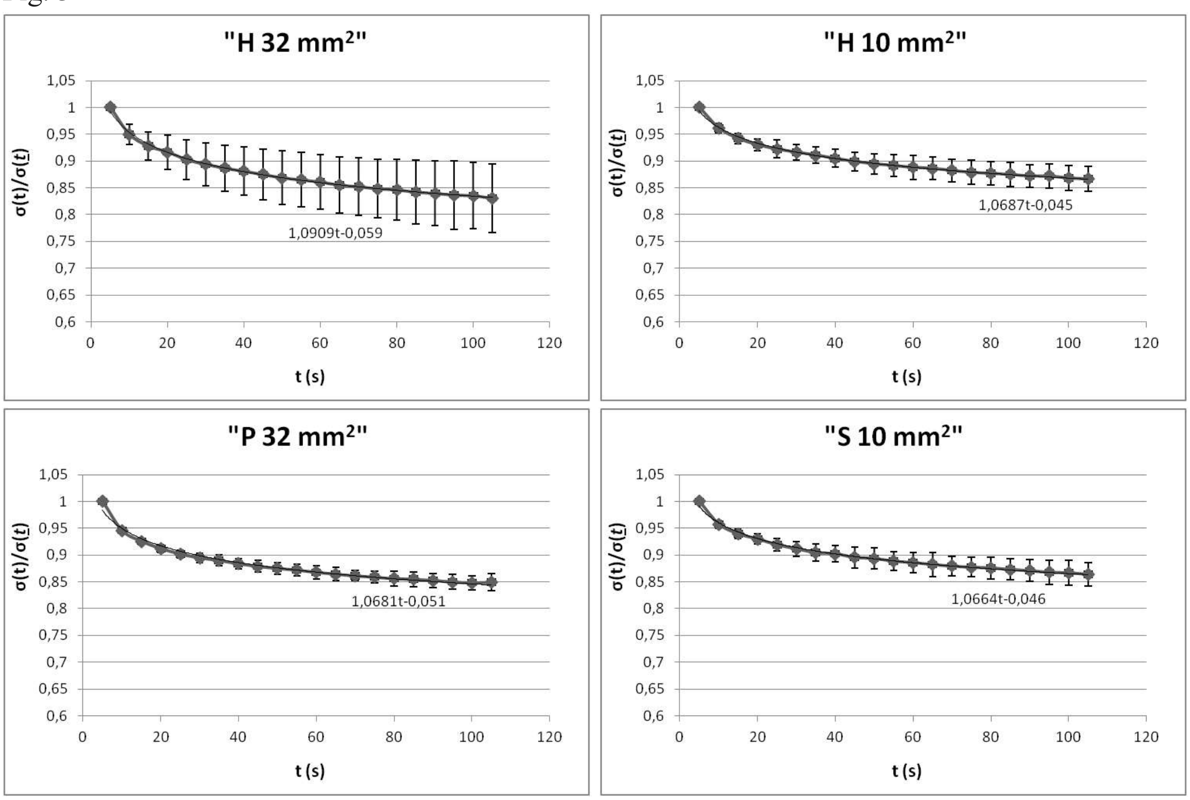

"H 32 mm2"
"H 10 mm2"
"P 32 mm2"
"S 10 mm2"
σ(t)/σ(t)
t (s)
1,0909t-0,059
1,0687t-0,045
1,0681t-0,051
1,0664t-0,046

Fig. 4

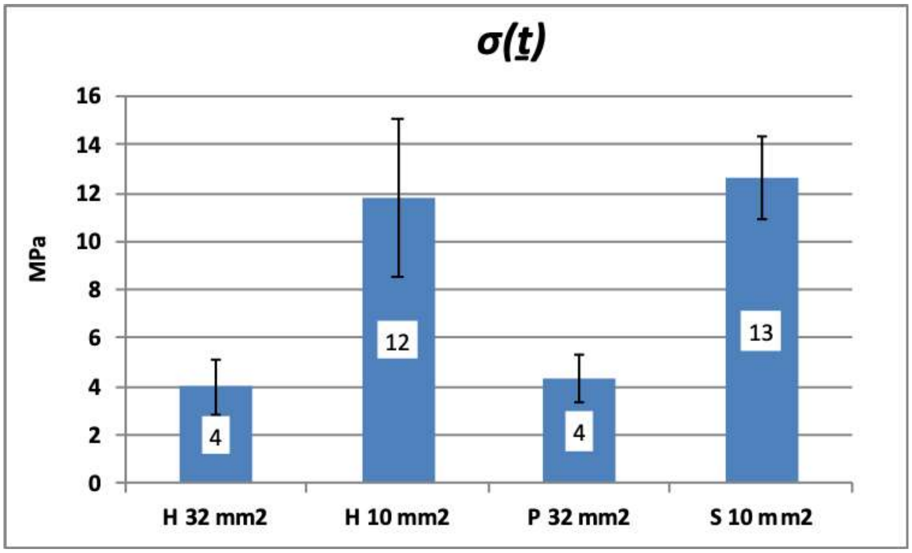


Fig. 5

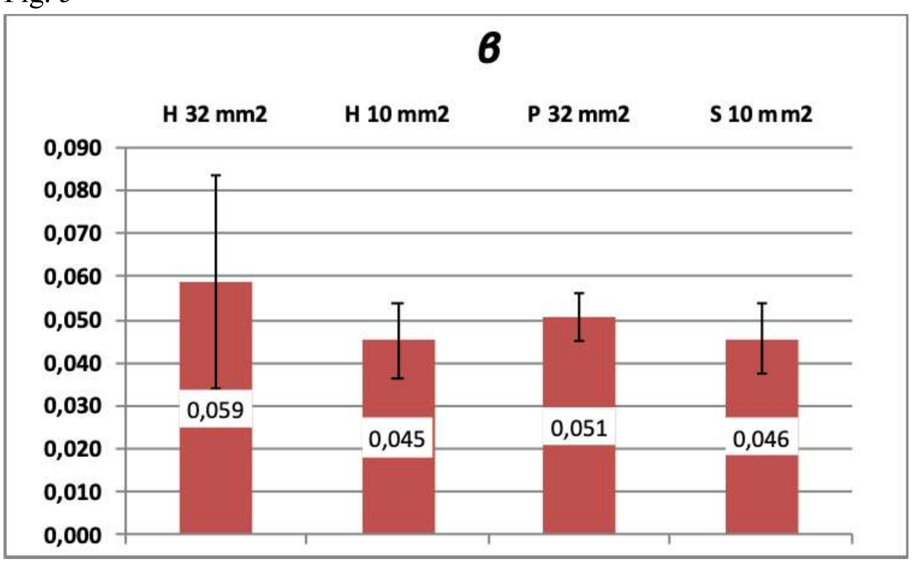
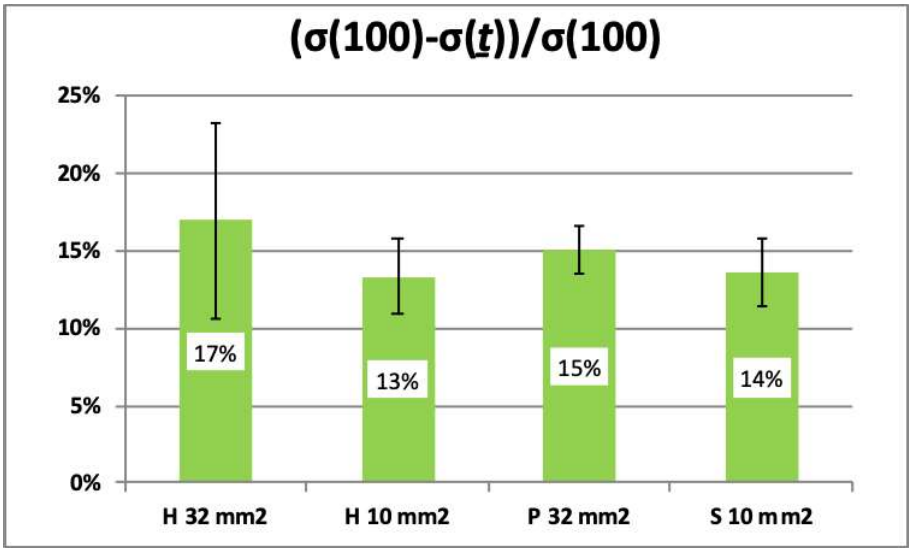


Fig. 6


(σ(100)-σ(t))/σ(100)
25%
20%
15%
10%
5%
0%
17%
13%
15%
14%
H 32 mm2
H 10 mm2
P 32 mm2
S 10 mm2

How preconditioning and pretensioning of grafts used in Anterior Cruciate Ligaments surgical reconstruction are influenced by their mechanical time-dependent characteristics: Can we optimize their initial loading state?

by

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Structured Abstract

Purpose: Consensus about a pre-implant preparation protocol adaptable to any graft used in Anterior Cruciate Ligament (ACL) reconstruction is still lacking. In fact, there is not agreement on reliable metrics that consider both specific graft dimensional characteristics, such as its diameter, and the inherent properties of its constitutive material, i.e. ligaments or tendons. Aim of the present study was to investigate and propose *the applied engineering stress* as a possible metrics.

Methods: Preconditioning and pretensioning protocol involved groups of grafts with different section (10 or 32 mm^2) and materials (i.e. human patellar and hamstring tendons, and synthetic grafts). A 140 N load was applied to the grafts and maintained for 100 s. Initial stress and following stress-relaxation (a mechanical characteristic that can be related to knee laxity) were specifically analysed.

Findings: Initial stress, ranging between 4 and 12 MPa, was affected primarily by the graft cross-section area and secondarily by the choice of the graft material. In terms of loss of the initial stress, stress-relaxation behaviour varied instead on a narrower range, namely 13-17%.

Interpretation: Engineering stress can be identified as the correct metrics to optimize the initial state of each graft to avoid excessive stiffness, laxity or fatigue rupture phenomena.

Keywords:

Anterior cruciate ligament; graft; preconditioning; pretensioning; stress relaxation

Introduction

Anterior Cruciate Ligament (ACL) is one of the major ligaments within the human knee; It plays a critical role in joint stability since it avoids lateral dislocations of the femur with respect to tibia. Injuries of ACL are very common due to its prominent role and alteration of knee biomechanics as well as early development of osteoarthritis (OA) are some of the consequences of ACL damage [40]. These considerations lead clinicians toward ACL surgery reconstructions, in the last decade [32] that however– despite reporting promising short- to mid-term functional performance [39] – are not fully able to prevent premature knee OA [15] as well as to a return of complete physical activities [34].

The paradigm at the basis of ACL reconstruction implies the use of grafts to mimic the functional behaviour of the native ligament since inappropriate graft properties may limit the long-term success of ACL reconstructions [17]. Indeed this is a critical issue that is usually avoided by preconditioning and pretensioning of the graft to eliminate tension and stiffness decay after final fixation, despite a validated preconditioning/pretensioning protocol is still lacking [28]. Some recent clinical protocols have been proposed by Zheng et al. [43], that, however is difficult to generalize to different graft diameter from those used by the authors. Recently the effects of the initial elongations on the relaxation of some kind of fibrous tissue has been studied in stochastic context to assess a numerical predictive model for the mechanical features of the fibrous tissues [3].

In order to gain a robust and shared consensus about time-dependent behaviour of grafts used in surgical reconstructions, as first, the correct definitions should be introduced and shared. In this case, despite *tension/stress* appears as the fundamental terms – and in fact we talk about *pretensioning* [43] and *stress relaxation* [28] –, current *preconditioning* protocols are carried out in terms of applied *force*, while *stress* depends on the graft section area, i.e. graft geometrical characteristics.

The first assumption of this study is that – focusing on *stress* – a preconditioning protocol can be optimized with respect to the graft section, i.e. diameter, since geometrical factors affect, significatively, the results of the protocol [3]. The second assumption is that the protocol tuning on the graft diameter may be not sufficient to obtain an optimal outcome, because the grafts involves different tissue with different mechanical behaviour, although of the same size.

In this experimental study these assumptions are investigated to provide the effects of an "in vitro" mechanical test, mimicking a simple preconditioning/pretensioning protocol, and apply it to several graft samples used in ACL reconstruction. Final aim of this work is to identify reliable metrics able to support the optimal tuning of the initial loading conditions for any graft chosen in the treatment of ACL injury.

Materials and methods

Specimen selection and preparation

The experimental campaign involved two different types of human tissues, namely patellar (P) and hamstring (H) tendons, and a commercial synthetic graft (S). This choice was justified by the fact that these tissues are commonly used in ACL reconstruction [18]. They were collected from a Tissue Bank (*Science Care*, USA). The study was approved by the local Ethics Committee (protocol "TISS-KNEE" 8425). Tendons were stored at -80 °C and thawed in a 37 °C water bath for 15 min prior to testing [16] and then prepared by an experienced orthopaedic surgeon. After preparation, each specimen was cleaned and cut at the same length, 2 cm, before clamping (Fig. 1). Four different groups were prepared:

- "H 32 mm^2", an H group with section 32±11 mm^2, with pretensioning load of 140±18 N that corresponds to 4 MPa stress, equivalent to the pretensioning load of 160 N used in [43] for 7 mm diameter grafts and indicated as good for stiffness and strength.
- "H 10 mm^2", an H group with section 10±11 mm^2, with pretensioning load of 140 N was applied.

- "P 32 mm^2", a P group with section 32±1 mm^2, with a pretensioning load of 140 N was applied.
- "S 10 mm^2", an S group as furnished by the manufacturer with section 10±1 mm^2, with pretensioning load of 140 N was applied.

Each group was composed of six samples, as indicated in [26].

Testing Procedure

The aim of this study is the definition of an "optimal" procedure to tune the initial loading conditions of grafts. The experimental set-up used to this aim has involved the use of a single-axis electroforce dynamic system (Bose 3330, TA instruments) to perform uniaxial tensile tests. Sample principal fibres were qualitatively aligned along the machine loading axis (Fig. 1.d). Clamping was obtained with the aid of milled grips, involving sandpaper for the synthetic grafts and a surgical basting suture at the end of hamstrings grafts (Fig. 1.a) [35]. In order to obtain reliable results, a specific experimental protocol consisting in four phases consecutively applied on the same sample was developed (Fig. 2):

1. *Preconditioning*: specimens were preconditioned by harmonic load between 20 and 100 N, for twenty cycles at 0.25 Hz, thus to remove any crimping in the tendon fibrils caused by prolonged storage in a fixed position [23].
2. *Recovery*: a 20 N load was maintained for 15 min to exhaust sample elongation. After recovery, the initial length of the specimen as well as its initial width and thickness were measured along three sections of mid-substance by using a standard digital caliper; measurements were repeated three times by two different operators and values were then averaged to calculate strain and engineering stress [25].
3. *Pretensioning*: a load was applied to induce pretension on the graft, considering a linear ramp with a loading rate of 315 N/s [13].

4. *Stress relaxation*: sample position reached in pretensioning was maintained for 100 s to mimic the behaviour of the grafts being implanted into the knee joint.

During the whole experimental test, specimens were continuously moistened with saline solution [22]. Stress relaxation lasted 100 s, that is an observation time chosen for accurate estimation of the phenomenon through a power-law model [33].

Data and Statistical Analysis

Mechanical characterization was obtained in terms of the engineering stress $\sigma(t)$, obtained from the experimental data as $\sigma(t) = F(t)/A_0$, where $F(t)$ is the force measured during the uniaxial test and A_0 is the average cross-sectional area measured at the end of the recovery phase.

We denoted $\sigma(\bar{t})$ as the stress at the end of loading ramp and the stress relaxation is measured in terms of the percentage decay with respect to the initial value with the introduction of the ratio:

$$\rho(t) = \frac{\sigma(t)}{\sigma(\bar{t})} = At^{-\beta} \tag{1}$$

where $0 \leq \beta \leq 1$ is a decaying rate, expressing the speed of relaxation, whereas A is a non-dimensional coefficient depending on the stress level $\sigma(\bar{t})$ as shown in forthcoming paper.

A specific comparison was carried out between the “H 32 mm^2” and “H 10 mm^2” groups to highlight the influence of graft diameter with the relaxation protocol reported in this study. Further, two more comparisons interested the “H 32 mm^2” and “P 32 mm^2” groups, and the “H 10 mm^2” and “S 10 mm^2”, respectively, to highlight the influence of graft materials on the decaying ratio. An additional comparison has been reported for specimens “P 32 mm^2” and “H 10 mm^2” to show that difference in relaxation may be considered strongly dependent on the specific material used but if different diameters are used then the data are affected by this choice.

In order to check the influence at 100 s of the different grafts used other comparisons were obtained in terms of pretension $\sigma(\bar{t})$, relaxation rate, β, and loading loss at 100 s introducing the loading loss ratio $r(100) = \frac{\sigma(\bar{t}) - \sigma(100)}{\sigma(\bar{t})} = 1 - A\, t^{-100\beta}$, and they have been performed by using *Wilcoxon-Ranksum test* with a level of statistical significance *p-value* = 0.05.

Results

The experimental data from the mechanical tests reporting the normalized stress with superimposed power-law fitting, are reported in Fig. 3.

The results about the pretension level $\sigma(\underline{t})$ are collected in Fig. 4. The observation of the data shows that with a three times larger section A_0, the "H 32 mm^2" the group presented about a three times lower $\sigma(\underline{t})$ respect to the "H 10 mm^2" set; difference was statistically insignificant (*p-value* 0.0248*)*. No difference was present between different materials with same section.

The relaxation rates β are collected in Fig.5. It can be observed that samples with larger section, in particular the "H 32 mm^2" set, shows faster relaxation as no statistical difference is present.

Loading loss, $r(100) = \frac{\sigma(\bar{t}) - \sigma(100)}{\sigma(\bar{t})} = 1 - A\, t^{-100\beta}$ (Fig. 6), is strictly connected with β, as expected, since the faster is the relaxation, the higher is the tension decay in a given time interval. Thus, the "H 32 mm^2" showed the highest decay, but no statistical difference is present.

Discussion

The first assumption about the influence of the *stress* in the role of the ACL graft cross-section area (i.e. diameter), was specifically demonstrated by comparing grafts of the same typology, i.e. hamstring tendons, including different section areas. Indeed, beside the influence of the cross-section area on the value of the stress, a specific influence on the rate of decay on the stress level have been observed.

The second assumption, about the importance of the graft tissue, arise from the need to tune the preconditioning/pretensioning protocol for each graft. However, the protocol did not show any significantly different effect depending on the graft typology at least for the selected test conditions and analysis. Indeed, as we compare the results of the starting protocol for similar condition a negligible difference in value of the decay order is observed. Such a comment yields that for clinical application only the value of the initial prestress may be considered when patellar or hamstring tendons are used as driving information for the surgery.

The proposed preconditioning and based-on-*stress* pretensioning protocol shows comparable values of graft loss of tension after fixation, with respect to literature [36][14]. These findings further underline the strong effect of pretensioning and hence the need to define a proper protocol in clinical applications. In this regard data provided in this study may be used as a guide for the surgeon to apply a specific value of the pretension force as the cross section of the graft has been measured before the replacement of ACL.

The influence of the graft cross-section has been observed primarily on pretension and also, with less extent on stress relaxation. In particular for H, the test yields an inverse relationship between graft area and pretensioning and a direct relationship between graft size and relaxation. The results obtained for P are in agreement with literature [1].

The pretensioning load influences the mechanics of the grafts to a large extent [43]. Stress, appears is the correct metric to take into account both the contributes, with the aim to identify an optimal approach for graft selection and preparation. Indeed, the inherent definition of stress could help comparing and exploiting the results obtained in different studies. For instance, indications about a "dangerous" pretension – i.e. an excessive applied force able to introduce alterations in structure –, seem to be different if we consider a load of 340 N for a hamstring graft [43] and a load of 500 N for a hamstring-polyethylene hybrid transplant [19]. Nevertheless, focusing on cross-section area, we found that the first study used a graft diameter of about 7 mm, whereas the second of about 9 mm, thus both leading to a common indication in terms of *stress* (for both about 8 MPa).

Such consideration is known in biomechanics since stress is fundamental in interpreting structural-mechanical relationship [5][7][6][8]. The previously reported stress value of 8 MPa represents an important basis also to interpret our results. While Hingorani et al. [27] and Vena et al. [41] showed that a higher pretension load led to a higher level of relaxation, the behaviour identified in this work appeared to be quite different, presenting lower level of relaxation when higher stress was applied; anyhow in [27] only pre-damage strains were used and it was speculated that a separate behaviour may be identified at higher levels of induced stress. This could be indeed our case, since the higher stress values are all above the critical value of 8 MPa, thus micro-damages could have impaired a full reorganization of the tissues and therefore the overall relaxation behaviour.

In the analysed testing conditions, the influence of different graft materials was not that evident. Despite few differences due to the proposed testing protocols, consistency with literature was specifically identified in tension level and rate of relaxation for both P and H samples [43][29][1][24][13]. Indeed unfortunately, no data for comparison were found in literature concerning synthetic graft, with particular attention to the specific polyethylene terephtalate material. The results involving relaxation of P,H and S grafts showed that:

1) The extent of relaxation rate is strongly influenced by the stress level in the order of decay β.
2) A quite similar biomechanical behaviour has been observed for the considered tendons P, H and the artificial graft S as prestress increment corresponds to lower load loss ratio at 100 s
3) The value of the decay's order β is similar for the considered tissues, P,H, S as the same value of pretension is applied to the specimen.

Despite this study did not find different macroscopic behaviours related to the several grafts tested, their specificity should not be ignored. Micro-structural differences were in fact reported between hamstring and patellar tendons [20][21]. Although these grafts are of the same typology of tissue, they were harvested from different knee anatomical locations, definitely with a specific different function. For this reason, under mechanical test conditions different from these observed in this study, some significant differences were highlighted [42]. For the analysed synthetic solution, differences with respect to hamstring were not attended in general, as hamstring graft represents a "golden standard", thanks to a broad range of available analyses [13][30][11][19]. Nevertheless, new materials are under development to obtain optimal ACL graft properties [2], therefore focusing on *stress* - here proposed as one possible tuning metrics - can be useful to identify the optimal graft diameter.

This study presented several limitations and that may be improved in the close future. Concerning testing, despite the use of several solutions to avoid slippage of the soft tissues, further experiments should necessarily consider the clamping performed on bone insertions or the use of special gripping techniques [38][4]. The obtained results show that the major limitation pertains to the small sample size and testing conditions, that did not allow us to completely generalize relationships between graft diameters and materials. Nevertheless, this study provided an important basis that can be used as a preliminary approach to optimize in particular the preconditioning/pretensioning protocol and, more in general, the choice of graft in the treatment of ACL injury.

Conclusions

A preconditioning and based-on-*stress* pretensioning protocol was proposed to forecast stress decrease of ACL grafts after fixation within the knee joint. *Stress*, defined as force on cross-section area, resulted a useful metric to optimize the surgical protocol and can be used to reach a consensus in the preconditioning/pretensioning approach depending on graft choice. Furthermore, the presented findings supported the necessity to analyse the static and time-dependent mechanical behaviour of standard and innovative graft solutions, to increase the basic knowledge about them with the perspective of obtaining optimal clinical and functional outcomes.

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Figure Captions:

Figure 1. Preparation for testing of: hamstring tendon sample H (a), patellar tendon samples P (b), synthetic ligament S (c). A P sample clamped in the traction machine (d).

Figure 2. Scheme of the testing protocol, where F is the vertical force read by the load cell.

Figure 3. Stress relaxation dimensionless mean curves (markers) for patellar P, hamstring H and synthetic S samples with section area 32 or 10 mm^2, and relative power fitting superimposed (straight lines). σ(t) stands for tension at time t, σ($\underline{t}$) is the tension at the beginning of the relaxation (pretension).

Figure 4. Pretension for patellar P, hamstring H and synthetic S samples with cross-sectional area of 32 or 10 mm^2.

Figure 5. Relaxation rate β for patellar P, hamstring H and synthetic S samples with cross-sectional area of 32 or 10 mm^2.

Figure 6. Loading loss at 100 s for patellar P, hamstring H and synthetic graft S samples with cross-sectional area of 32 or 10 mm^2.

Figures:

Fig. 1 a-d

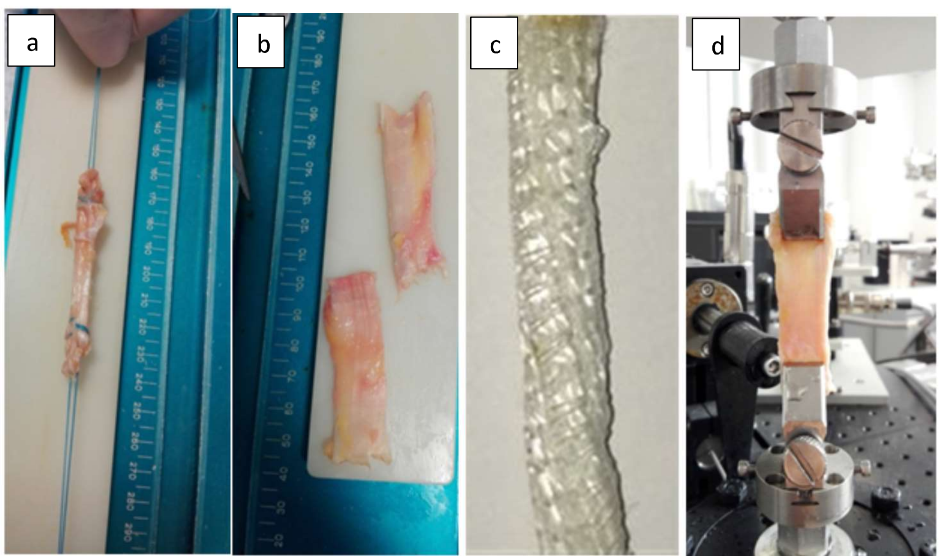

Fig. 2

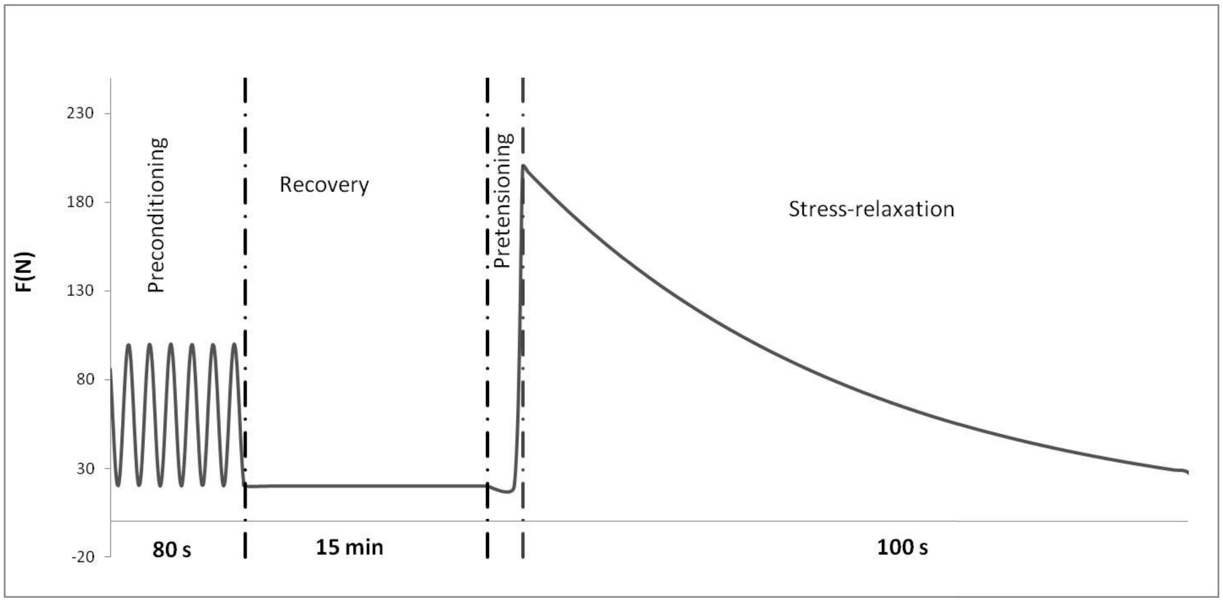
F(N)
230
180
130
80
30
-20
Preconditioning
Recovery
Pretensioning
Stress-relaxation
80 s
15 min
100 s

Fig. 3

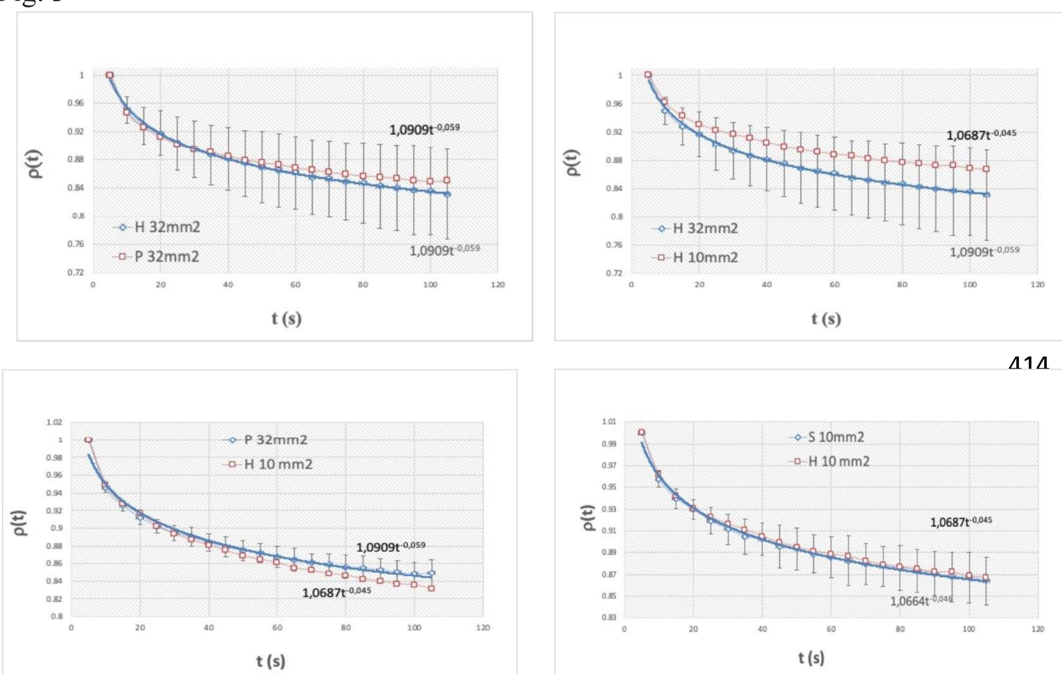
ρ(t)
t (s)
H 32mm2
P 32mm2
1,0909t^-0,059
H 32mm2
H 10mm2
1,0687t^-0,045
1,0909t^-0,059
P 32mm2
H 10 mm2
1,0909t^-0,059
1,0687t^-0,045
S 10mm2
H 10 mm2
1,0687t^-0,045
1,0664t^-0,046

Fig. 4

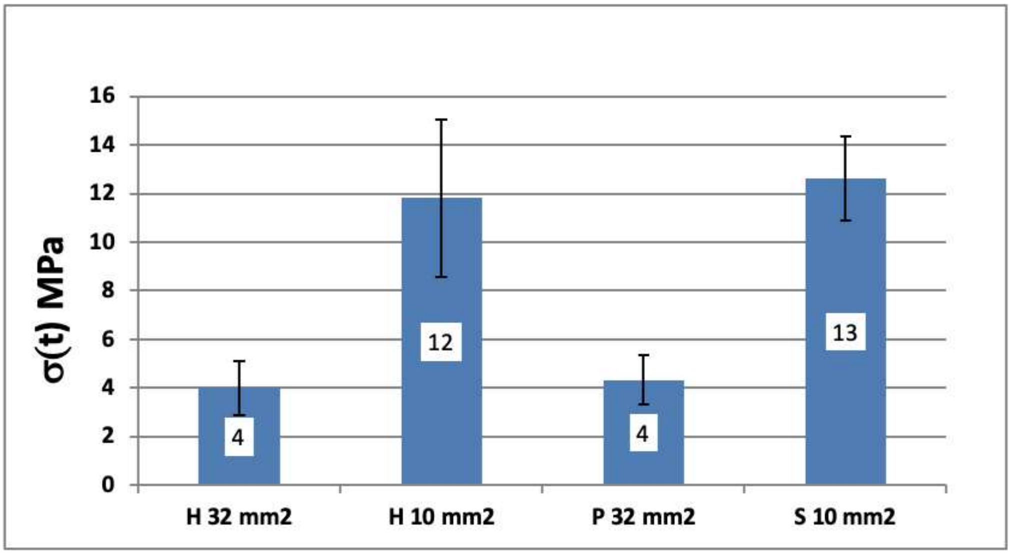
σ(t) MPa
16
14
12
10
8
6
4
2
0
4
12
4
13
H 32 mm2
H 10 mm2
P 32 mm2
S 10 mm2

Fig. 5

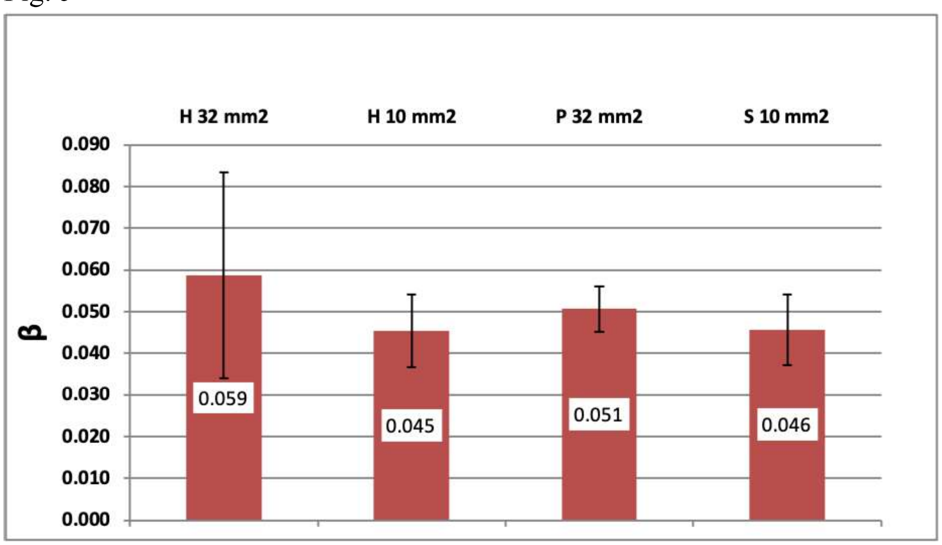
H 32 mm2
H 10 mm2
P 32 mm2
S 10 mm2
β
0.090
0.080
0.070
0.060
0.050
0.040
0.030
0.020
0.010
0.000
0.059
0.045
0.051
0.046

Fig. 6