Localised pressure stimulation using turf-like structures can improve skin perfusion in the foot

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Abstract:

Objective: Improving perfusion under the skin can potentially reduce ulceration and amputation risk in people with diabetic foot. Localised pressure stimulation has been proven capable of improving skin perfusion in the scalp but its effectiveness for the foot has not been tested. In this study, localised pressure stimulation was realised using flexible turf-like structures with dense vertical fibres and their ability to increase perfusion was assessed.

Methods: The skin in the rear-foot, mid-foot and forefoot of nine healthy volunteers was stimulated using two turf-like structures with different stiffness and one wound filler material that generated a uniform compression. Changes in perfusion were assessed using laser speckle.

Results: Mechanical stimulation significantly increased perfusion in the forefoot and mid-foot areas with the turf-like structures achieving higher and more long-lasting increase compared to the wound filler. The stiffer of the two turf-like structure appeared to be the most effective for the forefoot achieving a significant increase in perfusion that lasted for 25.5s immediately after stimulation.

Conclusion: The results of this study indicate that localised pressure stimulation is a more effective compared to uniform compression for improving skin perfusion in the healthy foot. Further research in people with diabetic foot disease is needed to verify the clinical value of the observed effect.

Keywords: Diabetic foot, foot orthoses, microcirculation, laser speckle, foot ulcer, wound healing.
Introduction:

More than 135 people per week have a limb amputated in the UK as a result of diabetic foot ulceration (DFU), but up to 80% of these amputations could have been prevented with correct management [1]. Based on current treatment costs for England, the clinical care of DFU and diabetic amputations costs between £0.97-1.13 billion annually [2].

Foot ulcers are linked to variety of clinical risk factors such as neuropathy and vascular insufficiency and biomechanical factors like increased plantar pressure [3,4]. More specifically, impaired microvascular perfusion and inadequate tissue oxygenation in diabetic foot disease are important risk factors for ulcer formation and ulcer healing [5–10]. Chuan et al. reported that ulcers in people with impaired lower limb perfusion tend to be wider, deeper and to develop more severe infections [5]. Therefore, assessing lower limb perfusion is crucial in the management of diabetic foot syndrome [11–13].

Assessing skin perfusion is an established and reliable method to study impaired lower limb blood flow [14–16]. Perfusion is a measurement of the rate at which blood is delivered to a tissue and usually is presented normalised over the area of interest [17]. Improving perfusion in the skin can potentially compensate for impaired lower limb perfusion and reduce ulceration risk [3,4]. Moreover, improving perfusion in a wound area, can improve wound healing rates [5].
There is an increasing awareness that insulin resistance and hyperglycemia in diabetic patients can induce microvascular abnormalities [18,19]. Furthermore, previous in-vivo studies indicated that plantar soft tissue in diabetic foot disease tends to be stiffer [20–22], which may also contribute to a lower skin blood flow [23].

While there is no established solution to improve blood perfusion under the skin in the plantar soft tissue or in a wound area, a number of methods has been identified to increase perfusion so far. Negative pressure wound therapy has been believed to increase blood perfusion in the wound area [24]; however, the method cannot be utilised on intact skin. At the same time negative pressure may cause pain and ischemia [24]. Extracorporeal shock wave therapy (ESWT) has been identified as an effective method to increase blood perfusion and microcirculation in patients with diabetic foot [25]. Despite the initial positive results, the use of this method is limited by the need for repeated applications in frequent intervals (=every two weeks), and the fact that it cannot be utilised outside the clinic.

To improve the perfusion in diabetic foot disease, Jan et al. investigated the effects of mechanical and thermal loading on perfusion [23]. They found that perfusion increases during rapid local heating stress at 42 °C and remains relatively constant under constant temperature. The results of this study also revealed that perfusion under the skin reduced during local mechanical stress at 300 mmHg and increased significantly just after unloading. The effect of mechanical stress reduced gradually after sudden increase in perfusion as a result of unloading. The specific loading conditions investigated in the study by Jan et al. involved sustained mechanical loading for three minutes which is significantly longer
compared to the loading during daily activities such as walking [26,27]. In order to improve blood perfusion in the foot and reduce ulceration risk, simple and safe methods for mechanical stimulation are needed that have a long-lasting effect and patients can use as a part of their daily activities.

Scalp massage with localised pressure points has been identified as a method to increase blood perfusion [28,29]. Therefore, similar biomechanical mechanism may contribute to increase the blood perfusion in the plantar soft tissue. To test this hypothesis, in this study turf-like structures (TLS) that can be 3D printed in the form of dense flexible fibres were utilised. This type of structure was used because it enables the development of localised pressure points and at the same time protects the foot from excessive levels of compression. More specifically, when the fibres are pressed against the plantar surface of the foot, localised pressure points are developed at their tips to stimulate the skin and, as compression intensifies, they buckle to offload the foot.

Skin is comprised of an outer thin layer of epidermis and inner thick layer of dermis [30]. The epidermis does not contain blood supply and microvascular and nerve networks are located in the dermis layer [30]. Consequently, any stimulation of the skin should affect the dermis layer. Therefore, the stiffness of the fibres should be stiff enough to stimulate dermis layer. On the other hand, very stiff fibres can cause damage by applying high force on a small area. Therefore, it is important to make a compromise between applying enough force to stimulate the dermis layer and avoiding harming the skin through applying high force. This study was conducted to assess the capability of the turf-like structures for stimulating the skin of the
plantar soft tissue. More specifically the effect of loading/stimulating the skin with this system on perfusion was measured.
Method:

Samples:

Turf-like structures were designed having vertical cylindrical fibres with rounded tips (Figure 1a). The fibres were 10 mm long and with a diameter of 0.6 mm. Samples had a square base with 121 fibres attached to it at distance of 2.5mm between each other. Two samples with the same design but with different stiffness were 3D printed using Polyjet technology. The softer one was printed using a single rubber like material with shore-A hardness of 16. To increase stiffness without changing the design and contact properties of the sample a second turf-like structure was printed having a rigid internal core (ABS) with a diameter of 0.025mm (Figure 1a). The mechanical response of the samples with internal (TLS-IC) and with no internal core (TLS-NC) was assessed in a series of mechanical tests. One commercially available wound filler (ABIGO Medical AB, Ekonomivagen, Sweden) was also included into the tests as reference. The wound filler consists of layers of fabric which bottom-out under minimal compression to create a flat contact surface with the foot and a uniform distribution of pressure. Comparison between the results for the turf like structures and the wound filler will enable separating the effect of localised mechanical stimulation from that of uniform compression.

The distance between adjacent fibres (2.5 mm) were decided based on closest proximity that would allow manufacturing through Polyjet 3D printing technology. The diameter of each fibre was calculated based on preliminary testing to ensure that the applied maximum pressure (i.e. maximum net force/ sample overall area) would remain significantly lower than
200 kPa which is considered to be a threshold for injurious loading in people with diabetic foot [31].

All mechanical tests were performed using a 3 kN INSTRON ElectroPuls™ E3000 load frame. Before testing, the samples were placed between the compression plates which were covered with sandpaper to prevent slippage of the fibres on the compression plates (Figure 1b). The samples were subjected to 20 load/unload cycles to 50% compression of the fibre’s length or wound filler’s height. Loading was applied at 20mm/s to simulate walking [26] while the imposed displacement, and the resulted reaction force were recorded at 100Hz. Temperature during testing was kept constant at 21.5°C (±1.5°C). Mechanical testing was repeated after the use of the samples for in-vivo testing to ensure that their mechanical behaviour did not change during the course of the study.

**In-vivo testing:**

Nine healthy participants (seven male, two female) with no history of foot pathology, average (±Stdev) age of 38 y (± 8 y) and average body mass of 76 kg (± 8 kg) were recruited for this study after necessary ethical approval was secured from the University’s ethics committee. All participants provided full informed consent prior to testing.

The participants were asked to stay in a room with controlled temperature (21.5°C±1.5°C) for 10 minutes before testing and then lied down on the couch in prone position. The acclimatisation for 10 min has been established to be adequate to allow the skin temperature...
to reach a constant value [32–34]. A Perimed Pericam laser speckle technique (Perimed AB, Jarfalla, Sweden) was utilised to measure blood perfusion on the forefoot, mid-foot and rear-foot of the right foot of the participants. The laser speckle is able to capture 100 images/second in a maximum area of 24cm * 24cm. The resolution of the device is 100 µm/pixel and the accuracy of measurements is ±3 PU.

A bespoke indentation device was utilised to stimulate each region of interests (ROI) with the different samples and skin perfusion was measured before and immediately after stimulation [20,35,36] (Figure 3). More specifically the indentation device stimulated, in separate tests, the rearfoot, midfoot and forefoot regions of the right foot of the participants with 10 load/unload cycles [35,36]. The loading/unloading rate was set at 20 mm/s to simulate walking [26]. The maximum displacement of the samples in compression was 40% of the height of the fibres or wound filler and the maximum force during stimulation was measured using load cell (Zemic load cell, L6E, C3). The skin perfusion was measured and compared before and after stimulation.

During stimulation, the field of view of the Perimed scanner was obstructed by the indenter. To enable the measurement of perfusion, the loading device was programmed to immediately retract the indenter at speed of 20 mm/sec once the stimulation protocol was completed. During this process the operator looked at the live stream of video in the Perimed software and clicked on the record button as soon as the complete area of interest was visible (and not obscured by the indenter head) in the sequence. The testing set-up did not have the capacity to accurately measure the time-lag between the end of stimulation and the start of
recording. However, considering the length of the backwards travel of the indenter it can be calculated that this was ≈1 sec. Standardizing the relative position of the indenter and the Perimed scanner ensured that the time-lag between the end of stimulation and start of recording, was the consistent across all participants.

The three samples (i.e. two turf-like structures and one wound filler) were tested in random order to cancel out any possible systematic effect of various biomechanical (e.g. tissue loading history) or physiological parameters (e.g. changes in heart rate, changes in blood pressure). Perfusion data were recorded at 94 images/s for the last minute before stimulation and the first minute immediately after stimulation.

After the end of the tests, the thickness of the epidermis layer at the tested areas, was measured using freehand ultrasound (SL2325, Esaote, Italy) to explore the effect of the thickness of the epidermis layer on the stimulation outcome [21].

Statistical analyses:

Preliminary analyses were performed to check for violations of the assumptions of linearity, normality and homoscedasticity. A between-subject analysis was performed to investigate if perfusion immediately after stimulation was significantly different than before stimulation. For this purpose, the measurements for the first minute immediately after stimulation were divided into intervals of 0.5 sec and the average perfusion measurement for each interval was calculated for each participant. These measurements were then compared against the
average perfusion for the last minute before stimulation. To avoid any bias in data analysis, the raw data was analysed by a researcher who was blinded to the participants’ ID and the types of materials that were utilised for the tests. The perfusion before intervention is expected to be practically constant, whereas the perfusion immediately after intervention was expected to gradually change [23]. For simplicity, the averaged perfusion for each 0.5 s time interval will be referred to, from this point on, as instantaneous average. Wilcoxon test of difference (statistical significance level=0.05) was used to assess the significance of differences between measurements before stimulation and all different time intervals after stimulation for all three ROIs.

A within-subject analysis was performed to assess the duration of the potential effect of stimulation on perfusion. For this purpose, the instantaneous measurements for each 0.5 s interval after stimulation were compared against the measurements for the last minute before stimulation using one way ANOVA (statistical significance level=0.05) with Bonferroni confidence interval adjustment.

Repeated measures ANOVA (statistical significance level=0.05) with Bonferroni confidence interval adjustment was used to assess the significance of differences between the three samples in terms of the duration of the effect of stimulation. The same statistical method was also used to investigate the difference between the thicknesses of the epidermis layer on the three different ROIs of the foot.
Results:

Samples:

The turf-like structures exhibited non-linear mechanical behaviour that changed rapidly in the first couple of loading cycles but gradually stabilised after that (Figure 4). During the first load cycle the turf-like structure had a relatively high initial stiffness until the buckling of fibres which led to substantial strain softening. Because of the relatively high loading rate, the fibres do not fully recover after the first load cycle which led to a substantial change in mechanical behaviour. From this point on, at the beginning of each loading cycle the fibres are already bent and the stiffness of the sample stays relatively low with no significant strain softening or hardening.

The wound filler had a relatively low stiffness at low strains which increased with strain (Figure 4a). Its mechanical behaviour remained constant between load cycles. Its force for 50% compression and its hysteresis factor were 12 N and 0.50 respectively.

A comparison between the two turf-like structures indicated that having an internal rigid core increased the stiffness of the sample as planned. More specifically the net force for 50% compression for the TLS-NC and TLS-IC was 14 N and 17 N respectively. Adding an internal core also slightly changed the visco-elastic properties of the sample. The hysteresis factor (the area between the loading and unloading graph/ the area under the loading graph) for the tenth cycle was 0.86 and 0.88 for the TLS-IC and TLS-NC sample respectively.
Repeating mechanical testing of all samples after the end of in vivo testing indicated that their mechanical behaviour was not significantly affected by repetitive loading. Indicatively, the force for 50% compression after the in vivo test for wound filler, TLS-IC and TLS-NC and wound filler were 11 N, 16 N and 13 N respectively.

**In-vivo testing:**

The median force which was applied during foot stimulation ranged between 6 N and 13 N. The magnitude of applied forces was consistent between samples for each ROI, but some differences were found between ROIs. More specifically the forces on the forefoot and mid-foot are significantly lower compare to the rear-foot area during stimulation with TLS-IC (Z= -2.666, P=0.008 and Z=-2.075, P=0.038 respectively). Furthermore, the force on the forefoot area is significantly higher compare d to the rear-foot area during stimulation with TLS-NC (Z= -2.666, P= 0.008) and during stimulation with wound filler (Z= -2.073, P= 0.038).

Preliminary analysis indicated that average perfusion before simulation and the instantaneous average perfusion after stimulation for the nine participants were not normally distributed. Therefore, Wilcoxon test of difference was utilised to investigate if perfusion after stimulation is significantly different than before stimulation. The results indicated that mechanical stimulation led to significant increase in perfusion in forefoot and mid-foot (Figure 5a.b). No significant change was observed in rear-foot (Figure 5c).

As it can be seen in figure 5, there is a clear trend for improved perfusion in the cases of forefoot and mid-foot following mechanical stimulation, with the turf-like structures
achieving higher increase compared to the wound filler. In the case of forefoot, significant increase in perfusion was achieved by both turf-like structures and by the wound filler (Figure 5a). More specifically, the average baseline perfusion for the wound filler, TLS-IC and TLS-NC was 29pu (±3pu), 27pu (±5pu) and 26pu (±2pu) respectively and its average increase during the first 0.5 s after stimulation was 66.3% (±36.0%) (Z=-2.666, P=0.008), 65.3% ± (39.0%) (Z=-2.547, P=0.011) and 72.8% ± (%22.5) (Z=-2.666, P=0.008) respectively. The final instantaneous average perfusion that was significantly different to baseline was 11.5 s, 25.5 s and 22.5 s after stimulation and the respective increase was 22.8% ± (22.0%) (Z=-2.192, P=0.028), 27.6% ± (29.8%) (Z=-2.073, P=0.038) and 19.8% ± (13.3%) (Z=-2.547, P=0.011).

In the case of mid-foot, significant increase in perfusion was achieved only by TLS-NC and the wound filler samples (Figure 5b). More specifically, the average baseline perfusion for the wound filler and TLS-NC was 33pu ± (7pu) and 29pu ± (5pu) respectively and its average increase during the first 0.5 s after stimulation was 31.5%±(21.1%) (Z=-2.429, P=0.015) and 66.8% ± (52.6%) (Z=-2.429, P=0.015) respectively. The last instantaneous average perfusion that was significantly different to was 2 s and 14 s after stimulation and the respective increase was 21.9% ± (28.6%) (Z=-2.073, P=0.038) and 41.2% ± (47.1%) (Z=-2.310, P=0.021). The time range for which instantaneous average perfusion after stimulation was significantly different to baseline can give a first indication of the duration of the effect of stimulation (Figure 5).

A better insight on the duration of the effect of mechanical stimulation on perfusion can be gained from the within-subject analysis of changes. One way ANOVA indicated that on
average the effect of stimulation at the forefoot was 32.3s (±22.7s), 26.3s (±18.4s) and 14.3 s (±8.1 s) for the TLS-IC, TLS-NC and the wound filler respectively. At mid-foot, the effect of stimulation lasted for 13.9s (±11.7s) and 13.7 s (±18.9 s) for TLS-NC and the wound filler respectively.

The duration of stimulation effect (effective time) was compared between different samples. One way repeated measures ANOVA with Bonferroni corrections showed that there is a significant difference between the effective time on forefoot area achieved by the TLS-IC (31.5 ± 6.0 s) versus the effective time of the wound filler (14.0 ± 2.5 s) (Wilks’ Lambda = 0.489, F(1.907,19.071) = 4.702, p=0.042). There is no significant difference on the effective time between three different tested samples on the mid-foot or rear-foot areas.

The measured thickness of the Epidermis layer on the forefoot, mid-foot and rear-foot areas were 0.64mm ± (0.04mm), 0.65mm ± (0.03mm), 0.85mm ± (0.03mm) respectively. One way repeated measures ANOVA with Bonferroni corrections showed that the thickness of the plantar soft tissue in rear-foot area is significantly higher compared to the thickness of the plantar soft tissue on forefoot and mid-foot area (Wilks’ Lambda = 0.205, F(2,16) = 7.089, p=0.029).
Discussion:

Diabetic foot ulceration is a severe complication in diabetic patients which is linked to a variety of clinical risk factors including vascular insufficiency [3]. Strong evidence from literature indicate that people with diabetic foot and impaired lower limb blood perfusion have higher risk for developing ulcers that do not heal and become infected [5]. This highlights the need for methods to improve blood perfusion in vulnerable areas of the foot in order to reduce the risk of amputation [3,4].

Mechanical stimulation with localised pressure points has been proven to improve blood perfusion in the skin of the head; however, the effect of localised pressure points on the blood perfusion in the foot has not been investigated yet. To replicate the effect of localised pressure points, turf-like structures were utilised in this study to stimulate the foot. A commercially available wound filler was also used as reference. Comparison between the perfusion before and immediately after stimulation for the turf-like structures and the wound filler enables separating the effect of localised pressure points (i.e. loading similar to scalp massage) from that of uniform compression.

The results of this study confirmed that, mechanical stimulation can increase perfusion in the foot. In a similar study, Jan et al. [23] reported significant increase in the perfusion underneath the first metatarsal head of 26 healthy and diabetic subjects. In the present study, significant increase in the average perfusion was observed immediately after stimulation at the forefoot and mid-foot areas.
The results of the current study showed that the same protocol for mechanical stimulation does not have the same effect on perfusion in different regions of the foot. More specifically the protocol presented here achieved a higher and more long-lasting improvement of perfusion in the forefoot than mid-foot. No significant improvement was observed in rear-foot.

In all cases the effect of the stimulation gradually decreased after exposure; with turf-like structures achieving, on average, higher and more long-lasting improvement compared to the wound filler material (Figure 5). In the case of longest effective time on the forefoot area, the TLS-IC improved the perfusion for 50 seconds; however, the improvement was statistically significant for the initial 25.5 seconds. Jan et al. [23] reported increase in the perfusion on the first metatarsal area for duration of almost 140 seconds after unloading (both for diabetic and healthy participants). Even though the effective time observed here appears to be lower compared to the one reported by Jan et al., differences in testing set-up and analysis protocols mean that the measurements of the two studies are not directly comparable to one another.

In terms of the type of mechanical stimulation, the protocol used by Jan et al. involved applying a uniform pressure that was sustained for three minutes [23]. In the present study a cyclic loading protocol that is closer to in vivo loading conditions such as walking was used
instead to show that localised pressure is a more effective method for stimulation compared to uniform pressure.

Compared to the duration of stimulation (i.e. 10 sec) the maximum duration of significant effect appears to be substantial (2.55 times greater). However, further research will be needed to investigate whether the duration of this positive effect on perfusion will increase with prolonged exposure to stimulation. At this point it has also to be highlighted that current literature doesn’t provide adequate evidence to support predictions about the duration or extent of increased perfusion that is needed to improve the prognosis of diabetic foot complications. Further research in people with diabetic foot disease is required to establish thresholds for clinically relevant improvement.

A between sample comparison indicated that the stiffer of the two turf-like samples (TLS-IC) achieved improvement of perfusion that lasted significantly longer compared to the wound filler in the forefoot area and therefore appears to be the most effective one for this particular area. However, the same sample was the only one not to achieve significant improvement of perfusion in the mid-foot area, indicating that different sample stiffness or intensity of stimulation might be needed to improve perfusion in different regions of the foot.

For the purpose of this study the design of the turf-like structures was decided mainly based on considerations about safety. Considering an overall area of 790 mm² the maximum average pressure (maximum bet force/ area) generated by the TLS-NC and TLS-IC turf-like
samples was 18kPa and 21kPa respectively; \( \approx 10\% \) of the potentially injurious loading [31]. The fact that measurable improvement in perfusion, relative to uniform compression, was observed for such low and therefore safe magnitudes of loading opens the way for further research in people with diabetes to optimise the design and mechanical properties of the turf-like structures.

In contrast to forefoot and mid-foot, no significant improvement of perfusion was achieved in rear-foot by any of the samples. The apparent insensitivity of rear-foot to the mechanical stimulation protocol used here could be explained by differences in the structure of the plantar soft tissue in the heel compared to the forefoot and mid-foot regions [37,38]. Moreover, it was found that the epidermis layer in the rear-foot was significantly thicker than the epidermis in the fore-foot and mid-foot areas. Considering the relatively low magnitude of applied forces, the increased thickness of epidermis could mean that samples only stimulated the outer layer of the skin (i.e. epidermis); whereas the capillaries that are part of the inner layer of skin (i.e. dermis) remained unaffected.

The motive behind this study is to explore methods for effective mechanical stimulation that can be used on a daily basis to improve skin perfusion in the diabetic foot and reduce the risk of amputation. According to literature, the magnitude of the applied compression was relatively low compared to everyday weight-bearing activities [23]. This indicates that the results of this study are more relevant to scenarios where stimulation is delivered by manually massaging the foot using a soft turf-like structure or by integrating the turf-like structure into footwear in conditions where plantar pressure is low i.e. during the terminal stance or the
swing phases of walking. However, the optimum method for stimulation delivery (e.g. in footwear during walking or as a short daily treatment etc.) can only be determined by the intensity and duration of stimulation that is needed to have clinically relevant effect on perfusion.

The role of reduced microcirculatory responses in the development of foot ulcer has been widely discussed in literature [39]. The hyperaemic responses are certain vasodilatory responses (immediate increase in skin blood flow), to stimuli such as local applied pressure, which play significant role in protecting the foot from ulceration [40]. Pressure Induced Vasodilation (PIV) is a protective cutaneous mechanism that relies on the excitation of cutaneous receptors in the skin in response to locally applied pressure [40]. It has also been established that PIV is impaired in people with diabetes and this is deemed to contribute to the decrease in the skin’s access to blood which induce local ischaemia and lead to the development of diabetic foot ulcers [41]. Hence the supervisor effect of localised pressure (compared to uniform pressure) in inducing vasodilation and immediate increase in the microcirculation, which was observed in this study, can potentially be useful in patients with diabetes to restore their skin microcirculation to a normal level.

At this stage the study was limited to healthy individuals as a necessary first step. The findings of this study will have to be verified for people with diabetes, while further testing will also be needed to assess the effect of prolonged stimulation on blood perfusion and on the outcome of diabetic foot disease. The fact that significant improvement of perfusion was achieved with the help of low magnitude compressive cyclic loading that does not have to be
sustained for prolonged periods opens the way for this type of research in large cohorts of people with diabetic foot complications.

Conclusions:

The results of this study indicate that localised pressure realised with the help of turf-like structures is a more effective stimulation method compared to uniform compression for improving plantar skin blood perfusion in the healthy foot. However, further research in people with diabetic foot and peripheral vascular disease is needed to verify the observed positive effect of localised pressure stimulation and identify the parameters for effective stimulation in different areas of the foot. Furthermore, this study focused on the immediate effects of brief mechanical stimulation on perfusion, however further research is needed to investigate the potential long-lasting effects of prolonged stimulation on perfusion.

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Fig 1.
Figure 1: The geometry of the turf-like samples (a) and the testing set-up for mechanical testing. The internal structure of the turf-like structure with internal core (TLS-IC) is also shown in detail.
Figure 2: In-vivo testing set up (bottom) and a typical graph of perfusion pressure as it was presented by the laser speckle system after the completion of the test (top).
Figure 3: The mechanical response of the three samples during the first, second, tenth and twentieth load/unload cycles. Results for the wound filler, the turf-like structure with internal core (TLS-IC) and the turf-like structure with no internal core (TLS-NC) are presented in separate graphs.
Figure 4: The effect of mechanical stimulation over time in (a) forefoot, (b) mid-foot and (c) rear-foot areas. The time at which perfusion after stimulation stops being statistically significantly different than before stimulation (p>0.05) is indicated with characteristic vertical lines.
Figure 5: Comparison between the applied forces during stimulation with different samples at the three regions of interest. Significant differences between samples are indicated with (*).
Figure 6: The results of the within subject analysis of the duration of significant improvement of perfusion after stimulation. Results are presented only for the cases where significant improvement was identified. Significant differences between samples are indicated with (*).

Figure 7: Comparison between the thickness of the epidermis layer in the forefoot, mid-foot and rear-foot areas. Significant difference between areas are indicated with (*).