Features of Lymphatic Dysfunction in Compressed Skin Tissues – Implications in Pressure Ulcer Aetiology

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Running title: Loading Affects Lymphatic Function

ABSTRACT

Objective

Impaired lymph formation and clearance has previously been proposed as a contributory factor in the development of pressure ulcers. The present study has been designed to trial fluorescence lymphangiography for establishing how lymphatic function is altered under a clinically relevant form of mechanical loading.

Methods

Lymph formation and clearance was traced in both forearms by an intradermal injection of indocyanine green (ICG)(50μl, 0.05%w/v), imaged using a commercial near-infrared fluorescence imaging unit (Fluobeam® 800). External uniaxial loading equivalent to a pressure of 60mmHg was applied for 45 minutes in one arm using a custom-built indenter.

Results

Loading was associated with a decreased frequency of normal directional drainage (DD) of ICG within delineated vessels, both immediately after loading and 45 minutes thereafter. Loading was also associated with non-directional dispersal (NDD) of ICG within the interstitium. Signal intensity within NDD was often greatest at areas of stress concentration, producing a 'halo pattern', corresponding to the rounded edges of the indenter.

Conclusions

These results suggest that loading skin with a clinically relevant magnitude of pressure alters both lymph formation and clearance. Further work to quantify impaired clearance under mechanical loading could provide valuable insight into their involvement in the development of pressure ulcers.

1. INTRODUCTION

Pressure ulcers (PUs) represent a long-term debilitating condition affecting a wide age range of the population, particularly individuals with impaired mobility in both hospital and community settings. Despite long-standing risk assessment scales and management strategies[9,11], the relative high incidence of PUs requires extensive treatment representing a significant financial burden on health services throughout the world[6]. Pressure ulcers are most commonly associated with the application of pressure over a bony prominence, typically generated during prolonged sitting or lying, weight bearing on a prosthetic or exerted by certain medical devices [3,8,19]. It has long been recognised that pressure ulcer aetiology may subsume a number of causative pathways. These include direct damage due to mechanical deformation, as well as impaired fluid transport in both blood and lymphatic vasculature in compressed tissues, along with and activation of additional mechanisms during reperfusion[4,15].

Interstitial fluid flux and lymphatic clearance in the skin serves to remove a number of potentially damaging substances from the interstitial space. Where lymph formation and clearance is impaired, these substances necessarily accumulate and can be expected to eventually contribute to soft tissue damage, with the potential development of PUs. Indeed, lymph stasis in the condition of lymphoedema and associated models has several demonstrable destructive manifestations, including inflammation, fibrosis and localised cell death [14,21,25]. However, it remains unclear whether comparable lymph stasis is produced under clinically relevant loading of soft tissues.

A substantial volume of evidence supports the view that dermal capillaries may collapse under clinically relevant magnitudes of uniaxial loading, leading to partial or total localised ischaemia [2,5,24]. By comparison, collapse of dermal lymphatic vessels under equivalent loading has only been demonstrated by two seminal studies by Miller and Seale[10,11], using the canine hind limb. They employed lymphoscintigraphy with Technetium-99m to assess lymphatic clearance directly under uniaxial loading using an indenter[10]. Their findings identified a critical pressure range

between 60mmHg (8kPa) and 75mmHg (10kPa), above which lymphatic clearance is impaired. It was interesting to note that pressures below 45mmHg (6.0kPa) produced an increase in the rate of lymphatic clearance. In a subsequent study, recovery of lymphatic clearance following 30 minutes of occlusion at 75mmHg was found to be highly dependent on the magnitude of the post-occlusive pressure[11]. Thus, minimal recovery of lymphatic clearance was reported when the pressure was reduced to 45mmHg (6kPa), whereas partial recovery was achieved when the applied pressure was completely removed for 30 minutes. However, adopting a similar approach using lymphoscintigraphy is contraindicated in humans due to the inevitable risks associated with radiation exposure to participants.

Near-infrared (NIR) optical imaging employing the fluorophore Indocyanine Green (ICG)[1] is recognised as a potential alternative to lymphoscintigraphy for assessing lymphatic function in a minimally invasive manner, with reduced associated risks. The procedure has already been successfully implemented in diverse medical fields ranging from ophthalmology to lymph node resection. Indeed, it has recently been employed in association with hydrostatic pressures applied to the lower limb of volunteers using a pressure cuff. The results indicated an occlusive threshold and thereby a mean (±SD) peak pumping pressure of 25.2 ± 16.7 mmHg in lymphatic collecting vessels[23]. Whilst several studies in humans and animal models have examined the effect of such hydrostatic pressure, both sustained and cyclic, on lymphatic function[7,13,22], this method of applying pressure deforms tissue differently to single-axis compression and has previously been demonstrated to produce a different physiological response [16]. Indeed, extreme hydrostatic pressures as experienced by deep sea divers do not cause soft tissue damage and thus do not represent an appropriate model for the conditions under which pressure ulcers typically develop.

The aim of this preliminary investigation was to explore lymphatic function directly under a relevant form of mechanical loading.

2. MATERIALS AND METHODS

2.1 MATERIALS

A detailed risk assessment was performed prior to submission for Ethics approval. Precautions were taken to minimise all risks to participants and the researcher, most notably the decision to administer ICG as a micro-dose, as has previously been reported[20]. The total dose of 0.05mg used in this study represents less than 1% of the typical intravenous adult dose of 0.1-0.5mg/kg body weight.

Ethics approval was obtained from the Faculty of Health Sciences Ethics Committee to recruit a small cohort of healthy participants for the study from the University of Southampton (REC ID:8309). All participants were provided with full details of the study prior to giving their informed consent. The participants were screened to exclude those presenting with:

- Contraindications for Indocyanine Green, particularly sensitivity or allergy to iodide.
- Any history of conditions known to affect the skin, lymphatics, liver, kidneys or the immune system.
- Current use of anti-inflammatory or vasoconstrictive medication which is systemic or topical to the test area.

8 participants were recruited, 2 female, 6 male, aged between 24 and 61 years. For all participants, test sites were located on the volar aspect of both forearms, with the allocation of 'test arm' and 'control arm' randomised for each participant.

2.2 METHODS

A micro-dose of ICG (50μL, 0.05%w/v) was injected at a shallow depth on both volar forearms, judged to be intradermal by resistance to injection and the visible raising of a bleb. Loading was applied on the test arm immediately after injection, centred on the injection site, using a specially designed loading mechanism with a built-in load sensor mounted on a cantilever beam (Figure 1). A nominal load equivalent to a pressure of 60mmHg (8.0kPa) was applied using a circular indenter of

diameter 42.4 mm, with curved edges to reduce stress concentrations. This pressure magnitude was maintained for a period of 45 minutes.

Upon removal of the load (T45), still images were captured in both loaded and unloaded control arms at five minute intervals, for a period of 45 minutes (ending T90). An established NIR camera system (Fluobeam® 800, Fluoptics, Grenoble, France) was used to image clearance of ICG by the localised lymphatic vessels. The system incorporates a spectrally confined (780nm-centered) laser light source, a sensitive camera and a longpass detection filter which restricts collection to >820nm. The system is well-suited for use with ICG, which exhibits a peak emission wavelength of approximately 845nm *in vivo*[12].

Figure 1. A photograph demonstrating the loading and imaging set-ups. The imaging head of the Fluobeam® 800 (right) was held by an articulated arm, thus allowing easy repositioning to image both arms. The indenter (left) was attached to a secure cantilever beam.

Due to direct imaging of the ICG deposit, a relatively short accumulation time of 20ms was prescribed to prevent excessive saturation of the image. For analysis, the resulting low-intensity

pixel values were multiplied (2-8x) using ImageJ[17], to delineate the vessels which were present. Signal pattern types were then described and their frequencies recorded for each image at both T45 and T90.

3. RESULTS

The dispersion of fluorescence signal from the deposit followed two distinguishable patterns, which were not mutually exclusive. The first, termed directional drainage (DD), is characterised by high signal intensity along a narrow path which is well-contained and is evident beyond the loaded area (Figure 2A). The direction of drainage was exclusively anatomically centripetal and fluorescence could be traced to the axilla by the end of the procedure (not shown). It is caused by complete drainage of ICG within a lymphatic vessel, as has previously been reported under the term 'Linear Pattern'[9]. Close examination reveals that the signal intensity varies along the path in a spatial manner, most probably coinciding with the locations of lymph boluses or 'packets'.

The second pattern, termed non-directional drainage (NDD), is defined by a poorly-confined dispersion of low-intensity signal from the ICG deposit (Figure 2B). A subset of NDD reveals an additional 'halo' pattern, which is characterised by a high-intensity signal in areas of inevitable stress concentration, specifically under the edges of the indenter, manifesting as complete or incomplete circles (Figure 2C).
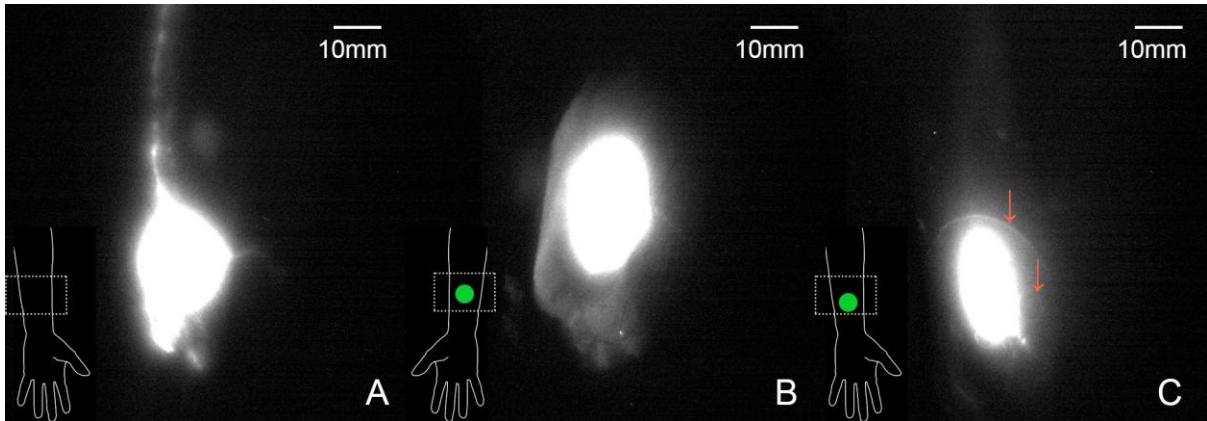
Figure 2. Typical images at T45 displaying the signal patterns directional drainage (DD)(A) in the control arm, and non-directional drainage (NDD) (B) and Halos (C), as indicated with red arrows, in the Loaded arm. White dots represent the area imaged. Loading is represented by a solid green circle.

Table 1 summarises the occurrence of patterns at both time points in arms where ICG was judged to be successfully injected at an intradermal depth, comprising 7/8 control arms and 7/8 loaded arms. At T45, DD was found to be present in both loaded and control arms. However, in loaded arms DD was typically confined to a single vessel of greatly reduced signal intensity, compared to control arms, with no evidence of lymph packets. Immediately following the reperfusion period at T90, DD signal intensity in loaded arms was increased, as additional vessels were delineated and lymph packets were defined. Additional vessels were also delineated at T90 in several control arms. The NDD pattern was primarily present in loaded arms at T45 and remained apparent at T90. The halo pattern was only present at T45 in loaded arms and present in association with NDD. At the end of the reperfusion period, the signal intensity of remaining halo patterns was greatly diminished.

| | Control | | Loaded | |
Pattern	T45	T90	T45	T90
DD	5	7	3	4
NDD	2	1	6	6
Halo	0	0	5	2

Table 1. A summary of signal pattern incidence in 7 Control and 7 Loaded arms, immediately after loading (T45) and after a recovery period of 45 minutes (T90).

4. DISCUSSION

NIR optical imaging with ICG represents a promising minimally-invasive technique for insight into lymphatic drainage in dermal tissues following the application of uniaxial compressive loading. As has previously been reported, the technique was associated with no serious complications, with some participants reporting only short-term pain upon injection and green tincture of the injection site lasting approximately 24 hours.

The results of this preliminary study suggest that uniaxial loading at an equivalent pressure of 60mmHg (8kPa) may represent a critical value for lymphatic function in the human dermis, where lymph formation and transport can become impaired in some able-bodied individuals but not others. These findings are in concert with a corresponding study in an animal model, where an equivalent pressure was found to obstruct dermal lymphatic clearance of a radiocolloid tracer in the hind limb of some but not all samples in the study [6].

Loss of directional drainage under loading may be caused by either collapse of lymphatic capillaries or elevation of lymph pressure, in such a manner as to oppose lymph formation. Delineation of vessels which do not exceed the area of direct loading (Figure 3) suggests that vessel collapse occurs primarily beneath the edges of the indenter. This might be predicted as, despite the curved edges, the pressure gradients within the tissue will necessarily be highest at these locations. However, it is also plausible that the lymphatic vessels were also collapsed under the entire area of the indenter, but re-fill immediately upon removal of the load prior to the onset of imaging.

Halo patterns, representing accumulation of the contrast agent, further demonstrate that peak mechanically-induced stresses/strains within the skin are associated with impaired interstitial fluid flow, as has previously been hypothesised[18].
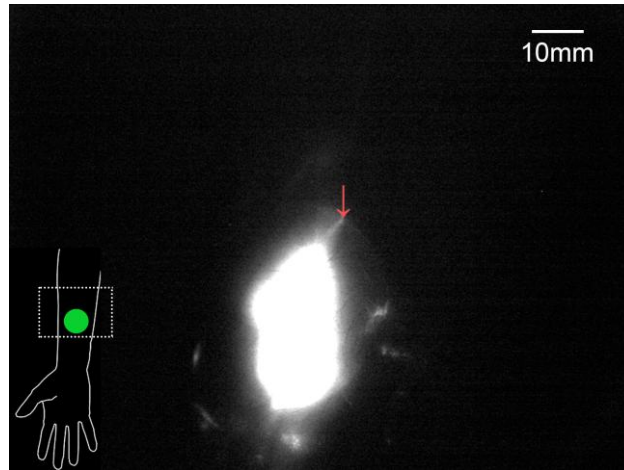
Figure 3. An image captured in the loaded arm, where vessels are delineated within the loaded area but not beyond the border of loading (red arrow). White dots represent the area imaged. Loading is represented by the solid green circle.

It is interesting to note that DD in loaded arms was noticeably different to DD in control arms at T45, although these differences were less evident following 45 minutes of reperfusion. This may indicate that the DD patterns present immediately after loading were not produced by active contractile clearance, but may instead be due to either passive clearance or the forcing of ICG into collecting vessels during initial tissue deformation. The effect of loading on the active propulsion of lymph will be addressed in future studies, using video data and fluorescence-signal tracking algorithms to quantify clearance of the ICG deposit.

This study was limited by sample size to a single magnitude of applied pressure for a fixed time period. Future studies should seek to establish a threshold pressure above 60mmHg, above which signs of lymphatic dysfunction including loss of DD occurs in all participants. By contrast, incidence of signal patterns may also be affected when the magnitude of loading is decreased. Indeed, the technique may provide additional insight regarding the reported increase in lymphatic clearance under application of low-magnitude pressures [8].

The technique described in this paper would be easily applied to other anatomical locations, including load-bearing sites associated with high risk of PUs. It could therefore provide an objective

measure for the effectiveness of support surfaces and periodic pressure relief strategies in protecting normal lymphatic function. With enhanced knowledge of the biological effects of loading at a range of magnitudes on relevant tissues, these interventions may be optimised to prevent the onset of tissue damage in individuals at risk of PUs.

5. CONCLUSIONS

In summary, this preliminary study represents the first of its kind to employ NIR optical imaging to indicate the functional state of lymphatic clearance in the human dermis following a period of uniaxial mechanical loading. This paper summarises the imaged manifestations of aberrant lymphatic function caused by this form of loading.

It is interesting to note that lymphatic dysfunction is indicated even in healthy volunteers, following acute loading at a magnitude equivalent to those pressures experienced in clinical settings during prolonged sitting and lying. Further research using the experimental approach may ultimately prove useful to inform the effectiveness of pressure management and relief strategies.

CONFLICTS OF INTEREST

None

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