NOVEL CHARACTERISATION OF ARTICULAR CARTILAGE IN THE EQUINE ATHLETIC JOINT

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We present a novel method of characterising articular cartilage using its free-swelling behaviour. We have attempted to quantify the site-specific matrix swelling properties of the third (C3) and radius carpal (Cr) cartilage from middle carpal joints obtained from 18 month-old Thoroughbred horses. The highly stressed dorsal region produced 63.7% more swelling than the lesser loaded palmar region (22.6% vs. 13.9%). For any given site there was a strong depth-dependence of swelling with the mid-zone being the highest ($p = 0.0002$). The technique was sufficiently sensitive to detect an increased swelling of +4.23% in sites with a mild lesion relative to those sites that were lesion free ($p = 0.04$).

KEYWORDS: equine middle carpal, articular cartilage, swelling, osteoarthritis, contact stress.

INTRODUCTION:

Articular cartilage has an intrinsic swelling potential generated by the hydrophilic proteoglycans (PG) constrained within the interconnected collagen fibril meshwork (Oloyede & Broom, 1994). The hydrated cartilage provides a deformable stress-attenuating layer covering the bone ends with the deformation response largely dependent on the outflow of fluid through the ultralow permeability matrix (Broom & Flachsmann, 2003). The amount and frequency of loading applied to the joint within the physiological limit results in positive adaptations in the extracellular matrix. However, loads beyond this limit are likely to initiate degenerative changes (Buckwalter, 2003). The common parameters used to measure changes in the matrix include collagen content and cross-linking, PG concentration and its aggregation size, water content and levels of various matrix marker proteins. However, quantification of such parameters is cumbersome and involves extensive biochemical processing of the tissues. By contrast, cartilage matrix swelling behaviour is a singular property that arises from the integration of all these components mentioned above and is readily observed experimentally. In this study, we present a novel technique to quantify swelling behaviour of the cartilage of the third carpal (C3) and radial carpal (Cr) bones of Thoroughbred horses and specifically focussing on sites of known higher versus lower stress.

METHODS:

Twelve healthy Thoroughbred horses were euthanized at 18 months and the middle carpal joints were harvested (Figure 1). Four adjacent sites (2 dorsal and 2 palmar sites) on the radial facets of C3 and Cr in the joint were marked with a temporary fuchsine stain to identify a consistent region of sampling. Sites 1 and 2 on the dorsal aspects of C3 and Cr corresponded to locations where high contact stresses, adaptive bone changes, a high incidence of osteoarthritis and osteochondral fracture have been reported {Pool, 1996 #27}. The lesser loaded palmar sites 3 and 4 were chosen for comparison. Similar sites 1 to 4 in the Cr were reproduced by mirroring the sites 1 to 4 on the corresponding C3.

Osteochondral blocks with *en face* dimensions of ~4×4 mm, were sawn from these four sites on each of the C3 and Cr bones and maintained in a fully hydrated condition (Figure 2). A special custom-made cutter produced a double-cruciform vertical cut of defined dimensions 2.2×2.2 mm through the full depth of the articular cartilage. Each block produced 30μm-thick serial tangential slices of initial en face dimensions 2.2 mm square using a sledging microtome. Each osteochondral block produced between 5 and 20 transverse serial slices depending on the cartilage thickness; this varied across the joint surface. The slices were fully hydrated in saline (0.15M) for 24 hours at 4ºC and then digitally photographed under

standard magnifying conditions in an optical microscope to determine the swollen in-plane dimensions relative to their initial dimensions (Figure 3a). Percentage areal swelling strains were calculated using Image J (Image J Image analysis software. 1.39d ed: National Institutes of Health, 2007) and linear mixed modelling was used to analyze the strain data (SAS V9.1, SAS Institute Inc., Cary, NC, USA).

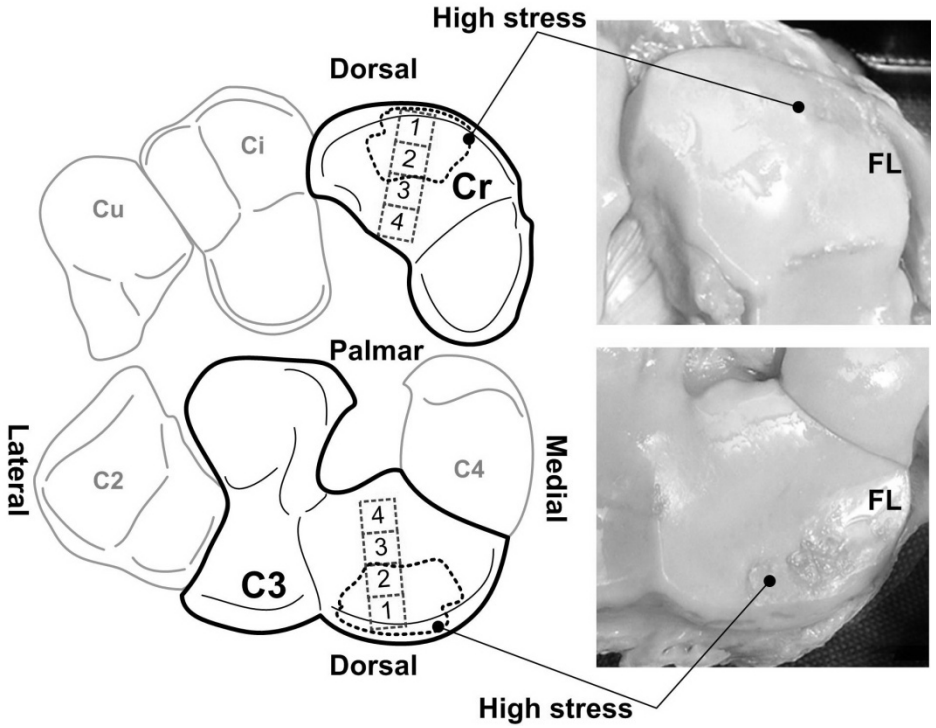

Figure 1 - Sites labelled 1-4 indicate sampling locations for cartilage matrix swelling studies on both C3 and matching Cr surfaces of the middle carpal joint. The boundary (-----) indicates the high contact stress region which incorporates sites 1 and 2. The two photographs on the right show focal lesions (FL) in this boundary region with significant damage to the joint cartilage.

RESULTS:

The cartilage matrix swelling strains from both C3 and Cr were combined and plotted as a overall mean site-specific swelling strain (figure 3b). Overall, the highly loaded sites 1 and 2 (dorsal region) produced higher swelling strains than the lesser loaded sites 3 and 4 (palmar region). Sites 1&2 and 3&4 produced mean swelling strains of 22.6% and 13.9% respectively. All sites exhibited a strong depth dependence of swelling yielding dome-shaped profiles in which the mid-zone produced the highest values ($p = 0.0002$). The technique was sensitive enough to detect mild lesions on the joint surface. Overall, a 4.2% increase in swelling was observed at sites incorporating a lesion relative to those that were lesion-free ($p = 0.04$).

DISCUSSION:

The first employment of swelling as a characterisation method for cartilage was performed by Maroudas (1976) in which she established the relationship that increased water content was proportional to the level of matrix degeneration (Maroudas, 1976). Since then, other investigators have performed similar studies (Bank et al., 1997; Setton et al., 1994). However, the method relies on measuring the increase in mass of the hydrated tissue, hence a minimum sample mass is required and any excess wetness may give false readings. In this new study we found overall that the swelling strain profile of each site showed a similar pattern to that reported in the earlier studies noted above. However our study provides enhanced swelling data resolution and higher sensitivity. Our measurement of

actual areal size increase has allowed significant miniaturisation of both the sample size (2mm vs. 7~10mm) and thickness (i.e. ~30μm vs. 250~1000μm). Overall, this improved the site resolution of the data as well as permitting swelling quantification of extremely thin cartilage (< 1mm) over sites with difficult contours. The miniaturisation also increased the sensitivity of the swelling results allowing the detection of mild cartilage matrix changes associated with lesion development. The technique also provides a method of characterising the cartilage matrix in relation to known patterns of loading in the middle carpal joint.

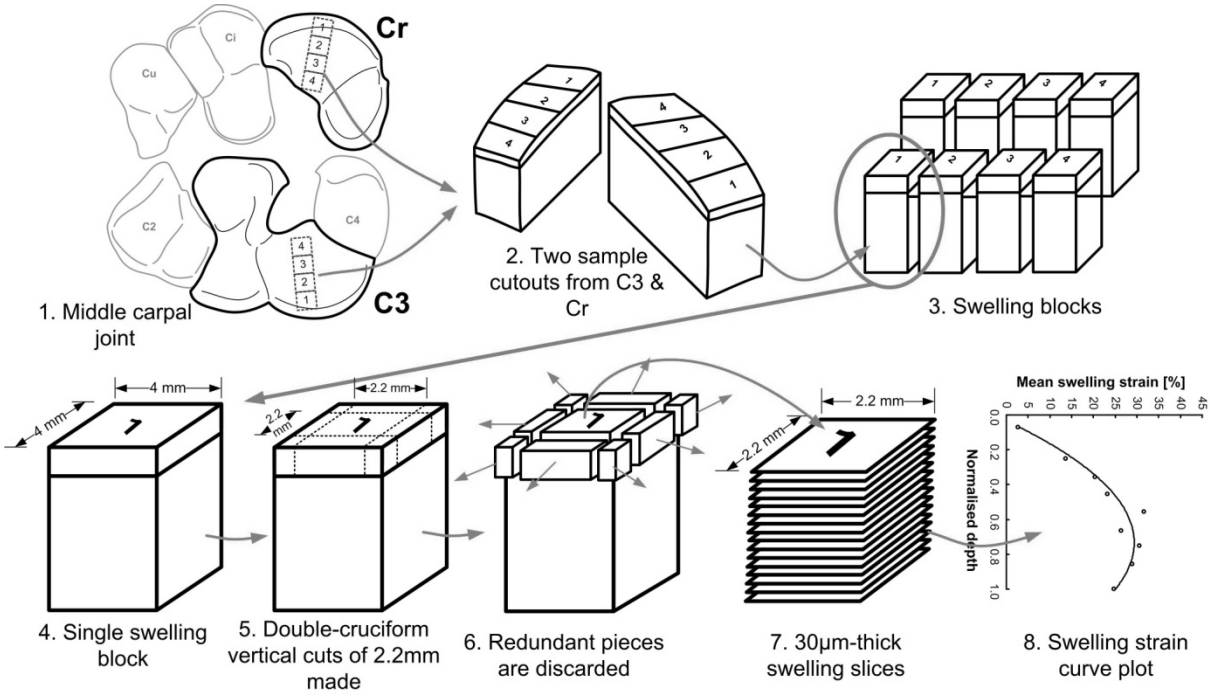

Figure 2 - Schematic showing procedure used for obtaining serial slices for the cartilage matrix free-swelling studies from the four sampling sites on the C3 and Cr surfaces.

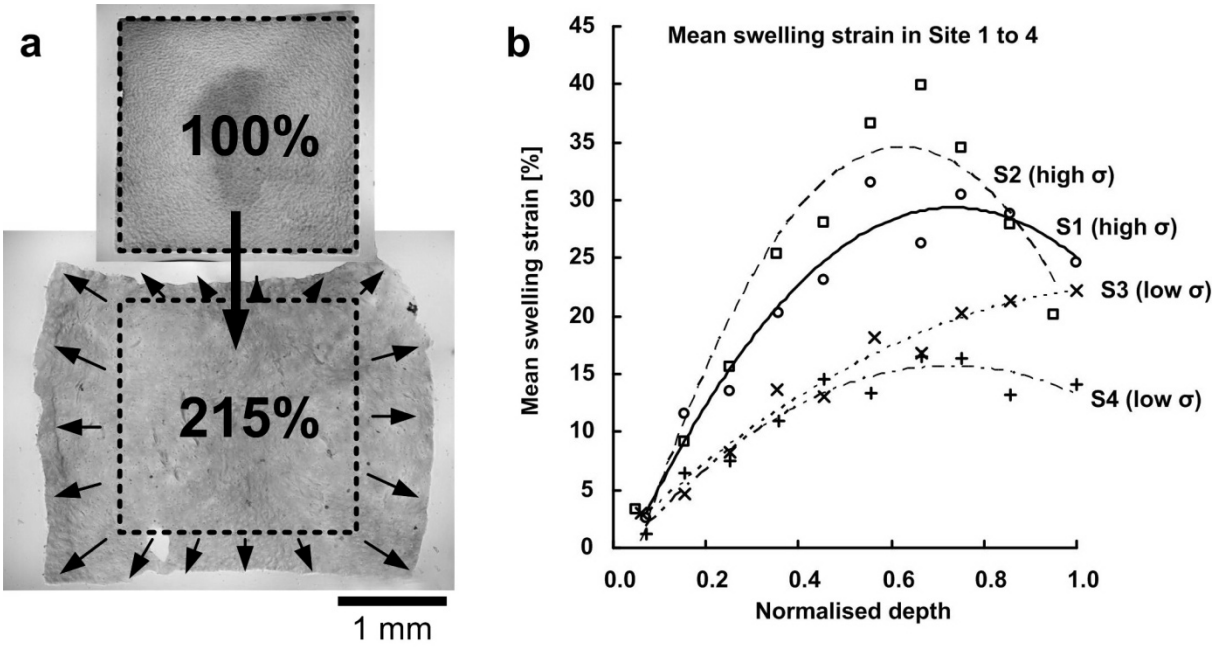

Figure 3 - (a) Examples illustrating matrix free-swelling behaviour in cartilage; (b) Mean matrix free-swelling strains over individual sites; Site 1(○), Site 2(□), Site 3(×) & Site 4(+).

CONCLUSION:
Our novel characterisation method uses cartilage matrix swelling behaviour to quantify changes in the extracellular matrix. The technique is able to differentiate site-specific loading variations as well as provide sensitive depth dependent swelling profiles at each site and with respect to the presence of mild lesions.

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