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COMPARISON OF THE ICRS VERSUS HHGS (MANKIN) CARTILAGE HISTOLOGY SCALES

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Purpose: Recently, the International Cartilage Repair Society (ICRS) has devised a visual histological scale for assessing cartilage repair at the sites of cartilage lesion. The ICRS scale assigns scores based on surface roughness, nature of repair tissue, cell distribution, cell population viability, and possible subchondral bone remodeling and cartilage mineralization at the site of repair. The values for this scale range from zero to eighteen, representing the full range from lack of repair to complete cartilage repair. This repair-specific scale has as yet seen very limited use. In the current study, scores assigned based on the ICRS visual histological assessment scale were compared to values assigned to the same images based on the more well established histological/histochemical grading scale (HHGS) devised by Mankin et al. While not specific to cartilage repair, the HHGS has been widely used to assess cartilage repair based on formation of repair tissue with normal tissue characteristics, including surface smoothness, cationic staining, cell distribution, and integrity of the tidemark.
Methods: Histology samples were collected from cadavers and (with prior IRB approval) from patients undergoing total joint arthroplasty. After de-calcification and paraffin embedding, 5 μm thick sections were stained with safranin-O/fast green and hematoxylin using standard techniques. Stained sections were then scanned with a microscope-mounted camera coupled with a stepper motor driven stage. A graphic user interface (GUI), developed in the widely used Matlab® programming environment, saved scores assigned by each observer (Fig. 1). Seven experienced human observers independently scored 30 images each. Linear regression was used to compare Mankin HHGS scores to ICRS scores.

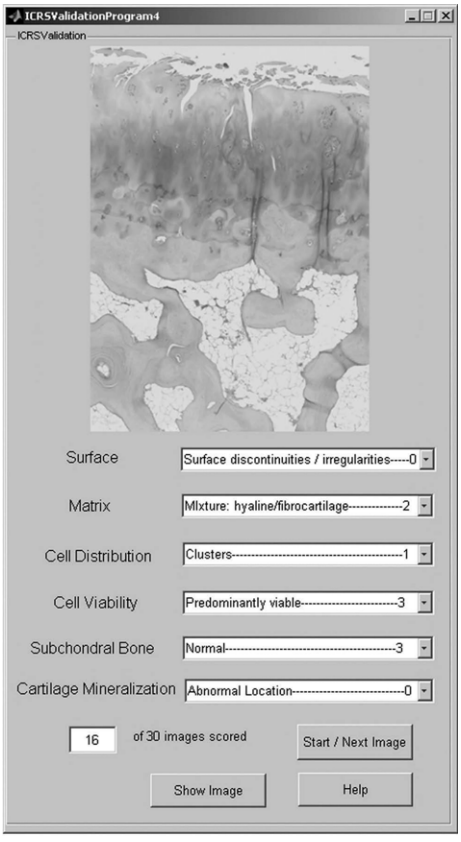

Figure 1. ICRS histology scoring graphic user interface.

Results: Comparisons between the HHGS scores and ICRS scores demonstrated a reasonable monotonic relationship, with the linear regression having a slope of -0.99, near to the ideal value of -1.29 (-18/14). In addition, the HHGS and ICRS values had a Pearson's correlation of -0.76, indicating a reasonably strong relationship (Fig. 2). Also, the absolute variance of the ICRS scores (25.54) was larger than that of the HHGS scores (12.94), but that difference was not statistically significant when the variances were normalized to their respective score ranges.

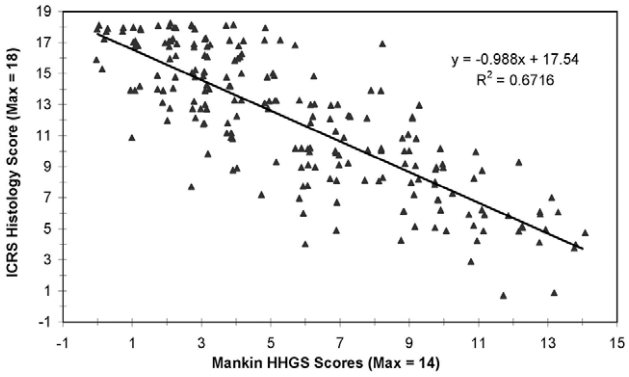

Figure 2. ICRS histology scores versus Mankin HHGS values. Data are plotted in "jitter" format (random noise superimposed), to allow visualization of individual data points for otherwise superimposed integer values.

Conclusions: The scored images did not specifically include sites of cartilage repair, but they exhibited the full range of features incorporated in the ICRS scale. While by no means above criticism, the HHGS is historically the most widely used cartilage histology scale, constituting a logical standard against which new assessments can be evaluated. The slope of the linear regression line between ICRS and HHGS deviated from its ideal value, indicating a higher tendency for cartilage to be rated more normal by ICRS than by HHGS. A possible explanation is that ICRS incorporates additional features not encompassed in HHGS, including changes in the subchondral bone and abnormal cartilage mineralization at the site of cartilage repair.

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OSMOLARITY INFLUENCES CHONDROCYTE DEATH IN WOUNDED ARTICULAR CARTILAGE

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Purpose: Mechanical injury results in chondrocyte death in articular cartilage. The objective of the study is to determine whether medium osmolarity affects chondrocyte death in wounded articular cartilage.
Methods: Osteochondral explants (n=48) harvested from the metacarpophalangeal joints of three-year old cows (N=6) were exposed to media with varying osmolarity (0-480 mOsm) for 90 seconds to allow *in situ* chondrocytes to respond to the altered osmotic environment. Explants were then wounded through the full thickness of articular cartilage with a fresh scalpel, incubated in the same media for 2.5 hours and transferred to 340 mOsm Dulbecco's Modified Eagle's Medium (DMEM, control medium) with further incubation for 7 days. Low power (x10) con-

focal laser scanning microscopy (CLSM) and fluorescent probes (Chloromethylfluorescein diacetate - stains the cytoplasm of live cells green, Propidium iodide - stains the nuclei of dead cells red) were used to determine *in situ* chondrocyte viability at 2.5 hours and 7 days. 'Z-sections' (optical CLSM sections with reference to the z-axis) were obtained perpendicular (imaging the superficial zone immediately below the articular surface) as well as parallel (imaging all zones within the full thickness of articular cartilage) to the wound edge. The spatial distribution of *in situ* chondrocyte death (zonal distribution), percentage cell death in the superficial zone (100 x (number of dead cells/number of dead and live cells) within a three dimensional reconstruction of the z-sections at the wound edge measuring 971μm(x-axis) x 200μm(y-axis) x 60μm(z-axis)) and marginal cell death (width of superficial zone chondrocyte death extending from the wound edge to the live/dead cell boundary) were compared as a function of osmolarity and time (2.5 hours vs. 7 days).

Results: *In situ* chondrocyte death was localised to the superficial zone of wounded articular cartilage for the range of medium osmolarities (0-480 mOsm) at 2.5 hours and 7 days, with relative sparing of the middle and deep zones. Compared to the control explants exposed to 340 mOsm DMEM, percentage cell death in the superficial zone was greatest for explants exposed to 0 mOsm (distilled water) and least for explants exposed to 480 mOsm DMEM at 2.5 hours (13.0% at 340 mOsm (control), 35.5% at 0 mOsm and 4.3% at 480 mOsm, $p<0.03$ for paired comparisons) and 7 days (9.9% at 340 mOsm (control), 37.7% at 0 mOsm and 3.5% at 480 mOsm, $p<0.01$ for paired comparisons). There was a significant decrease in marginal cell death for explants exposed to increasing medium osmolarity (0, 170, 340 and 480 mOsm), at 2.5 hours (mean margins 128.4μm, 22.1μm, 19.6μm and 8.6μm respectively, p=0.001, analysis of variance) and 7 days (mean margins 158.3μm, 42.1μm, 35.3μm and 11.7μm respectively, p=0.002). There was no significant change in percentage cell death from 2.5 hours to 7 days for explants initially exposed to any of the medium osmolarities.

Conclusions: Medium osmolarity significantly affects chondrocyte death in wounded articular cartilage. Greatest chondrocyte death occurs at 0 mOsm (distilled water). Conversely, a raised medium osmolarity (480 mOsm) is chondroprotective. The majority of cell death occurs within 2.5 hours, with no significant increase over 7 days. *Clinical relevance:* These experiments suggest that the osmolarity of irrigation solutions could be manipulated to reduce chondrocyte death from mechanical injury during arthroscopic and open articular surgery.

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DIFFERENCES IN INTRACELLULAR Na^+ AND K^+ CONCENTRATIONS BY SUPERFICIAL AND DEEP HUMAN ARTICULAR CHONDROCYTES

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Purpose: Condrocytes are sensitive to their ionic environment and respond with variations in the membrane transport proteins. Chondrocytes from different zones of the articular cartilage are known to have different properties. The aim of this study is to determine the [Na^+] and [K^+] intracellular by superficial and deep human cartilage.

Methods: Human cartilage plugs were cut into 20 microns slices with a microtome and ten consecutive slices were pooled, divideding the specimen into 200 microns sections starting from the articular surface. Each pool was rinsed in cold solution (SF3) for to stop the ionic transporters of the chondrocyte plasma membrane. The [Na^+] and [K^+] intracellular were measured by atomic absorption spectrophotometer after the removal of ions from the extracellular space.

Results: The table shows the [Na^+] and [K^+] intracellular values obtained. The values are mean$\pm$ e.s.m. of n experiments.The [Na^+] and [K^+] intracellular are expressed in nmol per mg of tissue.

	[Na+]	[K+]
Superficial-middle zone	33,8$\pm$2,9	40,8$\pm$3,1
Deep Zone	28,0$\pm$1,3	38,6$\pm$2,8
Calcified zone	26,8$\pm$0,7	35,8$\pm$1,9

Chondroytes recovered from the superficial and middle zone shows progressively more [Na^+] and [K^+] intracellular than chondrocytes recovered from deep and calcified zone of the same cartilage.

Conclusions: These data confirm the metabolic heterogeneity of human chondrocytes, and suggest that ions modulation may be involved of the turnover of the cartilaginous matrix in different zones of articular cartilage.

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EXTRACELLULAR NA+ CONCENTRATION IN HUMAN CARTILAGE: IMPLICATIONS FOR IGF, IL-1 AND TNFα SYNTHESIS BY CHONDROCYTES

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Purpose: Changes in extracellular matrix [Na^+] occur in OA. Previous results suggested that chondrocytes are sensitive to changes in their extracellular Na+ environment. Cytokines are important stimuli of the chondrocytes activation response that can accompany cartilage injury.

Here we studied the influence of extracellular [Na^+] on the synthesis of IGF, IL-1 and TNFα cytokines, in a human cultured chondrocytes.

Methods: Human chondrocytes were cultured in increasing extracellular [Na^+] (154, 175, 200, 225, 250, 275 and 300 mM). The biosynthesis of IGF, IL-1 and TNFα cytokines was examined by immunoprecipitation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the synthesized citokines: human chondrocytes cultured in increasing [Na^+] were immunoprecipitated with specific antibodies, loaded on polyacrylamide gels and processed for Western blotting using the corresponding antibodies. Quantification of Western-blots was performed and subsequent comparing of the intensity of the bands.

Results: In Western blots, the intensities of bands specific for IGF immunoprecipitated progressively increased as extracellular [Na^+] was increased. However, western blots revealed significantly smaller amounts of IL-1 and TNFα as the [Na^+] in cultured medium increased.

Conclusions: The chondrocytes exposed to high extracellular [Na^+] synthesised significantly more anabolic IGF cytokine and less catabolic IL-1 and TNFα cytokines. These observations demonstrate the critical role of extracellular [Na^+] in chondrocytes synthesis.