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Osteoarthritis and Cartilage

Review
Evaluation of histological scoring systems for tissue-engineered, repaired and osteoarthritic cartilage

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Summary

Objective: Regeneration of hyaline cartilage has been the focus of an increasing number of research groups around the world. One of the most important outcome measures in evaluation of its success is the histological quality of cartilaginous tissue. Currently, a variety of histological scoring systems is used to describe the quality of osteoarthritic, *in vivo* repaired or *in vitro* engineered tissue. This review aims to provide an overview of past and currently used histological scoring systems, in an effort to aid cartilage researchers in choosing adequate and validated cartilage histological scoring systems.

Methods: Histological scoring systems for analysis of osteoarthritic, tissue engineered and *in vivo* repaired cartilage were reviewed. The chronological development as well as the validity and practical applicability of the scoring systems is evaluated.

Results: The Histological-Histochemical Grading System (HHGS) or a HHGS-related score is most often used for evaluation of osteoarthritic cartilage, however the Osteoarthritis Research Society International (OARSI) Osteoarthritis Cartilage Histopathology Assessment System seems a valid alternative. The O'Driscoll score and the International Cartilage Repair Society (ICRS) II score may be used for *in vivo* repaired cartilage. The 'Bern score' seems most adequate for evaluation of *in vitro* engineered cartilage.

Conclusion: A great variety of histological scoring systems exists for analysis of osteoarthritic or normal, *in vivo* repaired or tissue-engineered cartilage, but only few have been validated. Use of these validated scores may considerably improve exchange of information necessary for advances in the field of cartilage regeneration.

Key words: Cartilage, Histology, Score, Osteoarthritis, Tissue engineering, Proteoglycans.

Introduction

The histological quality of cartilage is considered to be one of the most important outcome tools to objectify severity of cartilage pathology and success of its treatment[1–3]. Over the past decades, as knowledge on osteoarthritis (OA), *in vivo* repaired and tissue-engineered cartilage increased, the number of systems evaluating histological characteristics of these cartilaginous tissue types increased simultaneously.

One of the earliest systems for the grading of 'OA cartilage' was developed in 1949 by Collins and McElligott[4], who proposed a macroscopic system for classification of osteoarthritic changes of the human patella. This macroscopic system was succeeded by a microscopic system for analysis of OA cartilage by Mankin in 1971, who developed the now well-known Histological-Histochemical Grading System (HHGS[5]). From then, methods enabling study of cartilage characteristics *in vitro* as well as *in vivo* expanded, and in 1986 O'Driscoll *et al.*[6] presented a scoring system for analysis of a 'new' type of cartilaginous tissue, commonly referred to as '*in vivo* repaired' cartilage.

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The first report on successful cartilage regeneration in human clinical practice was presented in 1994 by Brittberg *et al.*[1], and the hyaline-like characteristics of the repaired tissue inspired researchers world-wide to further improve results from this procedure. Consequently, yet another category of cartilage histology developed, being that of the 'tissue engineered' cartilage.

While introduction of new scoring systems in all categories continued[7–9], and in part due to disadvantages of earlier systems[10,11], the Osteoarthritis Research Society International (OARSI) as well as the International Cartilage Repair Society (ICRS) established committees to develop standardized histological grading systems for qualitative description of various cartilage characteristics[8,12].

The variety of available scoring systems in the field of cartilage research may obscure the choice of the appropriate scoring system for a specific research setting. Further, it is often not clear which system should be applied to answer a specific research question, nor is it clear if a scoring system has been validated for this application. This may be important when comparing one's results to that of other cartilage researchers, or when aiming at validating methods to analyze proteoglycan content of cartilage non-invasively[13].

This review provides an overview of existing histological scores for evaluation of osteoarthritic, *in vivo* repaired, and *in vitro* regenerated cartilage, and discusses validity and applicability of each of these systems (Fig. 1).

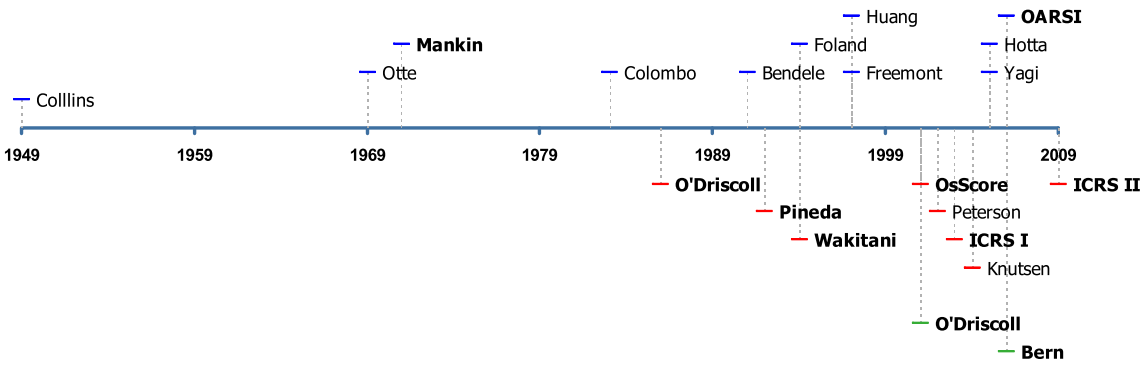

Fig. 1. Chronological development of cartilage histological scores after the first macroscopical score in 1949 (Collins). OA scores are presented in blue, *in vivo* cartilage repair scores in red, and *in vitro* tissue engineering scores in green.

Methodology in histological scoring of cartilage

A system for evaluation of cartilage histological properties should be comprehensive but also applicable to researchers with only basic knowledge of (cartilage) histology, regardless of the tissue type analysed. Intra- as well as inter-observer variation should be low and the score system is preferably validated. The importance of various characteristics for the ideal cartilage scoring system when developing a scoring system for *in vivo* repaired cartilage was recognized by Pritzker and was summarized as 'simplicity, utility, scalability, extendibility and comparability'[12]. Further, each scoring system should be used for its designated tissue type: scoring systems for OA cartilage should focus on degenerative features of healthy or diseased (OA) tissue, scoring systems for *in vivo* cartilage repair should focus on the degree in which a cartilage defect is successfully repaired, and scoring systems for tissue-engineered cartilage should focus on the quality of newly generated cartilage after *in vitro* cartilage tissue engineering.

Before choosing which scoring system is to be used, some basic knowledge of tissue pre-treatment and subsequent staining characteristics is required. To enable visualization of cartilaginous tissue, an ethanol–formaldehyde fixation most adequately preserves morphology[14]. Glycosaminoglycans are best visualized using a 'Safranin-O' staining[15]. When used in combination with a 'Fast-Green FCF/Hematoxylin' counterstaining, collagens and cell nuclei may be visualized as well. The 'Alcian Blue' staining also stains glycosaminoglycans, but has been reported to yield less reproducible results. In combination with a 'Picrosirius Red' staining, the Alcian Blue staining may visualize collagenous structures[16]. For both the Safranin O as well as the Alcian Blue staining, incorporation of control cartilage samples during the histochemical staining process prevents over- or underestimation of the glycosaminoglycan content due to variation in fixation and staining circumstances.

A variety of tissue characteristics may be assessed by a scoring system after histological staining of the tissue. Each characteristic may be scored separately in a quantitative fashion, or in combination with other characteristics in a categorical fashion, which may influence the statistical design and evaluation of the experiment. In a quantitative score, features considered important by the designer of the score like 'structure of the tissue' or 'proteoglycan content', may be emphasized through a higher contribution to the eventual score[5]. The resulting score may thus constitute the sum of the individual parameters, for example: a tissue is scored as five out of a maximum of eight points, if two points are obtained for 'intensity of Safranin-O staining', two points for 'cellularity', and one point for 'structural integrity'. The score may also be the result of multiplication of the abovementioned sum of parameters with 'stage' (extent of the involved cartilage surface)[12], thus obtaining a score describing the entire tissue area. Quantitative scores with a broad numerical range like the O'Driscoll score for *in vivo* repaired cartilage[17], may increase the likelihood of finding statistically significant differences between different groups, but may also be susceptible to a larger inter-observer variation.

Only few of the currently existing scoring systems have been 'validated'. Using a validated scoring system improves reliability of observations and improves comparability of these observations with results from other research groups. It is therefore important to know not only *if* a score has been validated, but also *how* the 'validation' was performed. Validation of a score may occur through comparison to already validated macroscopic scoring systems[11], to other already validated histological systems[18], to automated (and validated) histomorphometric systems[9] or to biochemical parameters[7]. Of these validation methods, correlation to proteoglycan content ('biochemical parameters') is often considered important, as proteoglycan content is considered one of the major features of cartilage integrity in healthy, repaired or tissue-engineered cartilage. Despite the number of scoring systems, only the HHGS for OA cartilage and the Bern score for tissue-engineered cartilage have been validated by biochemical analysis[5,7].

Vice versa, existing histological scores have been used as a tool to promote or validate emerging techniques analysing cartilage quality, as for example radiographic measurements[19], magnetic resonance imaging (MRI)[20,21] and new T2 and T1 contrast-enhanced cartilage imaging techniques as for example 'delayed Gadolinium enhanced MRI of cartilage' (dGEMRIC)[22].

All 'semi-quantitative' histological scoring systems are observer-dependent and thus subjective. Automated computerized histomorphometry has been reported to enable objective, accurate and reproducible analysis of cartilage characteristics. However, use of computerized histomorphometry may be limited by the high costs or by lack of technical expertise[9,23].

This review was based on a search for literature in which histological scoring systems were introduced, applied or discussed. PubMed, Embase and Cochrane Library were used for the search (syntax: *histological AND* (*scoring**

OR grading OR assessment* OR scale*) AND cartilage*. Last search: March 31st, 2009).

Osteoarthritis (OA)

HHGS/MANKIN SCORE

The HHGS or Mankin score for evaluation of osteoarthritic cartilage was originally proposed by Mankin in 1971[5], and although developed for the assessment of human articular cartilage it has also been used for grading of animal cartilage. There is much confusion about the use of the 'Mankin score', 'HHGS' or 'modified Mankin score' (the last of which there are many). As Mankin in 1971 referred to his score as the 'HHGS', we have decided to do the same. All other systems should be referred to as a 'modified Mankin system' or a 'modified HHGS' with specific remarks on the altered parameters.

The HHGS identifies 'cartilage structure', 'cell distribution', 'Safranin-O staining' and 'tidemark integrity' as separate subitems. The sum of the separate scores ranges from 0 (normal) to 14 (severe OA). The HHGS was correlated to a macroscopical score[11], to biochemical parameters and to $^{35}SO_4$ incorporation[5]. Although frequently used, the HHGS score has been criticized for its questionable reproducibility and inadequate assessment of 'mild' and 'moderate' OA[10,11]. Further, a lower score may result from scoring features like 'pannus' and 'surface irregularities', characteristics which may also be found in healthy, non-osteoarthritic tissue[10]. Moreover, the system does not evaluate the extent to which the cartilage surface is affected by the degenerative process[12,18]. As cartilage often contains different regions with varying quality, omitting the aspect of 'extent' may over- or under-estimate the overall quality of the tissue. Moreover, the HHGS was demonstrated to have a significant intra- and inter-observer variability[10,11,18,24] (Table I).

OARSI OSTEOARTHRITIS CARTILAGE HISTOPATHOLOGY ASSESSMENT SYSTEM

With the objective to design a more useful method than the HHGS to assess OA histopathology for wide application in clinical and experimental OA assessment, the OARSI Working Group developed the OARSI Osteoarthritis Cartilage Histopathology Assessment System[12]. The OARSI system assesses the severity and the extent of cartilage surface involvement in the local osteoarthritic process. In contrast to the HHGS and most other OA scores, the OARSI system emphasizes the extent of cartilage damage over the articular surface through a 'stage' component, in addition to damage analysed at several levels of the cartilage layer (i.e., 'depth' and 'local cartilage damage'). 'Grade' (0 points for 'normal' up to six points for 'severe') and 'stage' (0 points for 'no OA activity seen' up to four points for '>50% of articular surface affected') can be used separately or can be combined in an overall score by multiplication. The OARSI system was anticipated to be more adequate for the assessment of mild OA and it was expected that the OARSI system could be applied more consistently by less experienced observers than the HHGS[12]. In a study comparing the OARSI system with the HHGS, a similar reproducibility was found, however the reliability of the OARSI system was higher and indeed observer experience seemed to be less important when using the OARSI system[18]. Nevertheless, the authors reported that the staging component of the OARSI system was difficult to determine with certainty due to the precision required for estimation of the surface extent of osteoarthritic lesions[12]. This is demonstrated in [Fig. 2(B)], where interpretation of 'surface extent' depends on which 'grade' is considered most important i.e., the irregular area in the superficial zone of [Fig. 2(B)] is of a higher 'grade' (grade 2.0) but present in only 10–25% of the surface (stage 2) while areas with cell death (lower 'grade', i.e., grade 1.5) are present in more than 25% of the cartilage surface (higher 'stage', i.e., Stage 3). Although we used the OARSI system to illustrate differences in outcome between HHGS and OARSI, the OARSI system has not yet been validated for application on *human* articular cartilage, nor has it been validated through correlation to macroscopic or biochemical parameters [Fig. 2(A–C)].

OTHER OA SCORES

Besides the HHGS and the OARSI system, several other simple systems have been used to grade OA[19,25–31]. These scores are often referred to as 'modified Mankin' systems. In essence, all of these systems assess similar parameters as the HHGS, but the parameters are either scored in a different fashion, or an overall score is applied instead of separate subscores. Most of these scores were only used in the study in which they were introduced, and none were validated (Table II).

Cartilage repair

O'DRISCOLL SCORE

The first 'cartilage repair' score was used to assess the quality of cartilage repair in rabbits after periosteal grafting

Table I
Intra- and inter-observer variation of the HHGS

Year	Author	Assessment	Result	Comment
'92	Van der Sluijs *et al.*[24]	Intra- and inter-observer variation	Adequate	More strict criteria do not lead to higher reproducibility
'97	Ostergaard *et al.*[10]	Intra- and inter-observer variation; validated against macroscopical (Collins) score	Inadequate	HHGS inadequately discriminates between macroscopically normal and OA cartilage
'99	Ostergaard *et al.*[11]	Intra- and inter-observer variation; validation according to macroscopical appearance	Moderate	HHGS is valid for normal and severe OA cartilage, not for macroscopically mild and moderate changes. Experience not important.
'07	Custers *et al.*[18]	Intra- and inter-observer variation; comparison to OARSI system	Excellent	Reproducibility is experience-dependent

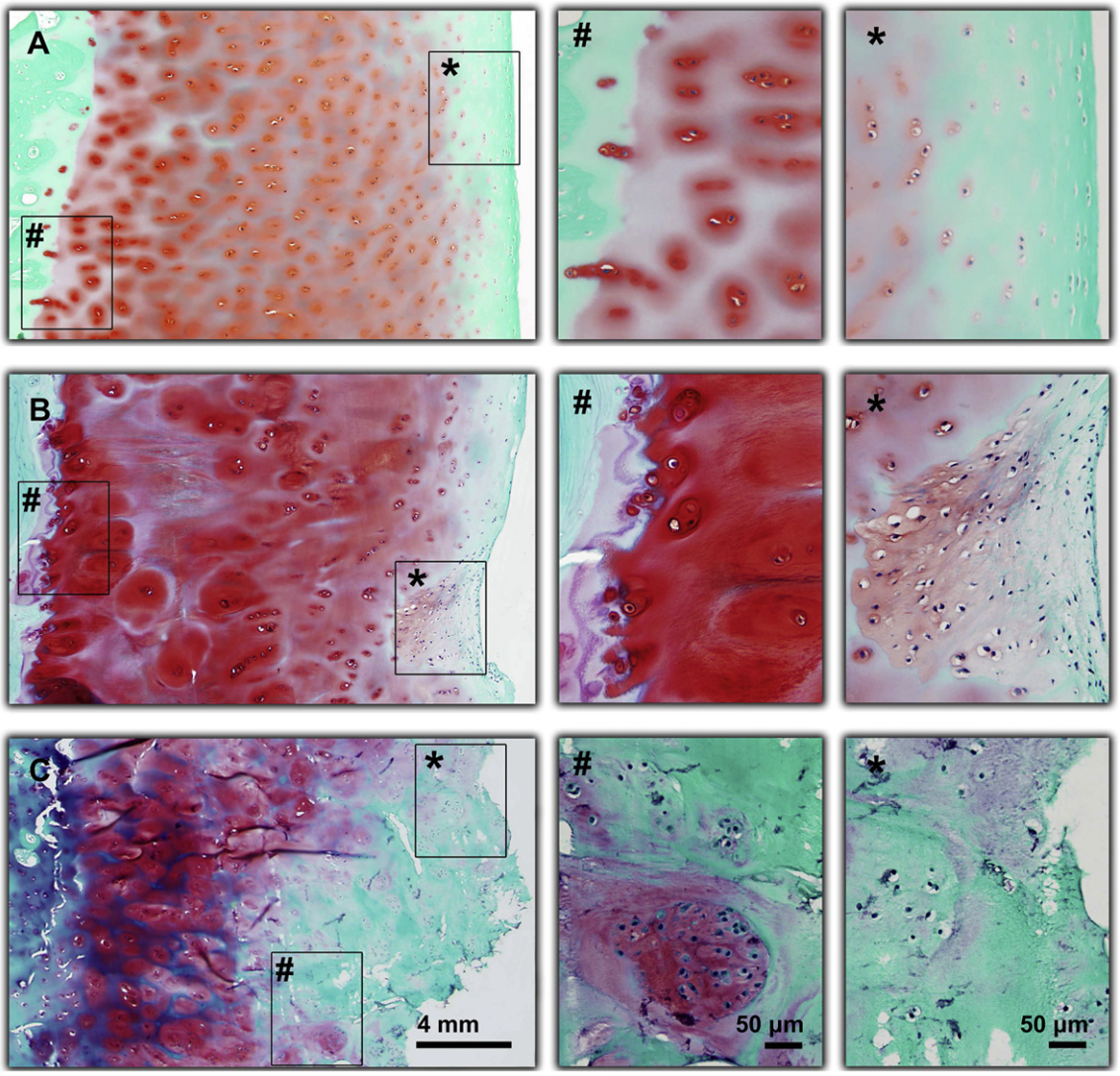

Fig. 2. Safranin O/Fast Green-stained histological sections of human knee articular cartilage (femoral condyle, 5 μm sections). Sections were scored with the HHGS[5] and with the OARSI scale[12] in small (left) and larger magnification (right images, # and *). (A) Good quality cartilage of a 45-year old male tissue donor without history of traumatic or inflammatory joint diseases. The surface is smooth without irregularities (*), and the osteochondral junction is intact (#). (B) Moderate quality cartilage of a 57 year old female, obtained during an unicompartmental joint replacement. Note the altered structure of the cartilage, the slight surface irregularities (*) and the onset of cell clone formation (#). (C) Low quality cartilage obtained during a total knee replacement with fibrous tissue characteristics, demonstrating matrix loss, surface abrasions (*) and extensive cloning (#).

of cartilage trauma[6,17]. This score specific for cartilage repair, which is now often referred to as the O'Driscoll score, encompassed four major categories ('nature of the predominant tissue', 'structural characteristics', 'freedom from cellular changes of degeneration' and 'freedom from degenerative changes in adjacent cartilage') and was frequently used for cartilage analysis in animal studies[32–34]. A high intra- and inter-observer reproducibility was demonstrated, when used to analyze goat articular cartilage[35]. However, many different subitems make it a somewhat lengthy score. The O'Driscoll score is one of the few scores in which 'integration' of the repair tissue with its surroundings is assessed [Fig. 3(A, B)], however the additionally added (or subtracted) points do not greatly affect the eventual score. The O'Driscoll score has frequently been used in modified versions (Table III).

PINEDA SCALE

In an effort to create a more compact score to evaluate cartilage repair, Pineda *et al.*[36] developed a new system which was applied to assess the natural healing capacity of defects drilled into rabbit knee articular cartilage. The Pineda scale contains four categories: 'percent filling of the defect', 'reconstitution of osteochondral junction', 'matrix staining' and 'cell morphology'. Although it was hypothesized that a simple score as the Pineda scale would have a higher reproducibility than the O'Driscoll score, the more comprehensive O'Driscoll score demonstrated a similar intra- and inter-observer variation. The correlation with the Pineda scale was high (correlation coefficient of 0.71)[35].

A commonly used modification of the Pineda scale was introduced in 1994 by Wakitani *et al.*[37] to study the

Table II
Modifications of the HHGS and other OA scores

Year	Authors	Comment
'69	Otte[30]	Also known as 'Fassbender'; modified by Saal *et al.*[19]. Only grades cartilage surface structure. High correlation with 'surface structure' category of HHGS
'83	Colombo *et al.*[26]	The Colombo score is similar to the HHGS and was modified by Hotta *et al.*[67]
'87–'07	Several[68–82]	Varying modifications, however 'tidemark integrity' is a frequently discussed or excluded category
'91–'97	Bendele and Hulman[25], Fremont *et al.*[28], Huang *et al.*[29]	These scores directly apply an overall score of severity to the cartilage section. Bendele includes a staging component. Huang classifies into 4 grades: Grade II, for example, comprises flaking, superficial fibrillation, chondrocyte enlargement and hyalinization.
'94	Foland *et al.*[27]	Measures fibrillation, chondrocyte necrosis, chondrone formation and focal loss of cells. Modified by Frisbie *et al.*[83].
'05	Yagi *et al.*[31]	Scores matrix depletion and cellularity quantitatively

characteristics of repaired full-thickness cartilage defects in rabbits. This 'Wakitani score' assesses 'surface regularity', 'thickness of reparative cartilage compared with surrounding cartilage' and 'integration of donor cartilage with adjacent cartilage', instead of 'percent filling of the defect' and 'reconstitution of osteochondral junction' in the O'Driscoll score. Both the Pineda and Wakitani score have been modified frequently[38–44], however solely the Pineda scale was validated[35].

THE OSWESTRY SCORE

In contrast to animal studies, study of articular cartilage repair in humans is limited by availability and size of the biopsy of the repaired tissue. For example, the category 'bonding to the adjacent cartilage' (O'Driscoll score) may only be scored when a biopsy is taken from a transitional zone between 'new' and 'old' cartilage, requiring either a large biopsy or a full joint explant. This issue was recognized by Roberts *et al.*[20], and as harvesting of large size biopsies including this transitional zone is usually not desirable as this may affect repair of the damaged area, the authors proposed a grading system for small biopsies of repaired human cartilage. The subsequently developed 'OsScore' comprises seven parameters ('tissue morphology', 'matrix staining', 'surface architecture', 'chondrocyte clusters', 'mineral', 'blood vessels' and 'basal integration') and has a maximum score of 10 points. The OsScore demonstrated a high inter-observer variability, and an excellent correlation with the O'Driscoll score ($r = 0.9$)[20].

KNUTSEN SCORE

In addition to these scoring systems, less extensive scoring systems were used for histological analysis of cartilage repair. Knutsen *et al.*[2] categorized cartilage biopsy samples using a hematoxylin–eosin staining and describes categories as 'hyaline' (≥60% hyaline cartilage), 'fibrocartilage–hyaline mixture' (40–60% hyaline cartilage), 'fibrocartilage' (≥60% fibrocartilaginous tissue) or as a sample 'without repair tissue'. In 2000 Peterson *et al.*[45], using multiple staining methods, used a similar system to evaluate the results of the autologous chondrocyte implantation (ACI), describing the tissue as 'hyaline-like', 'fibrous' or 'mixed'. Neither of these scores were validated.

ICRS

Interestingly, the ICRS Histological Endpoint Committee introduced the ICRS Visual Histology Score in 2003, arguing that for more easy and reliable comparison of histological assessment a system based on visual patterns is preferable over a verbal description-based system[8]. A web-based catalogue of cartilage repair images was set up as a reference for scoring, and to enable discrimination of the individually scored features, instead of summarizing all the individual subscores (I–VI) to create a total score, the score values (0–3) for the different categories ('surface', 'matrix', 'cell distribution', 'cell population', 'subchondral bone', 'cartilage mineralization') are described in the final score (i.e., I:3; II:3; III:1; IV:2; V:1; VI:3). To our knowledge no studies exist that have evaluated the validity and reliability of the ICRS Visual Histology Score. However, the OsScore and the ICRS score were found to correlate fairly well[46]. The ICRS VHS was modified by Chang *et al.*[47], by addition of categories for the staining of type I collagen and type II collagen.

A new histological scoring system has been developed and validated by the Histology Working Group of the ICRS[48,49]. This "ICRS II" score addresses various aspects of cartilage repair and was first applied clinically in a large prospective randomized trial in which the clinical and histological results of microfracture and ACI were compared[3]. The ICRS II score contains several categories which are subdivided in 13 categories each scored on a 100-mm visual analogue scale (VAS). A 100-mm VAS scale enables evaluation of subtle differences and facilitates statistical comparison of the individual cartilage characteristics[50]. The categories of the ICRS II score are, besides 'overall assessment' (bad–good), 'matrix-staining metachromasia' (no metachromasia – full metachromasia), 'tissue morphology' (fibril presence viewed under polarized light; full-thickness collagen fibres – normal cartilage birefringence), 'chondrocytes clusters' (four or more grouped cells; absent–present), 'calcification front/tidemark' (no calcification front–tidemark), 'subchondral bone abnormalities/cellular infiltration' (abnormal–normal/no infiltrates), 'architecture of the surface' (delamination/loosening/disruptions–smooth surface), 'basal integration' (no basal integration–basal integration), 'cell morphology' (no round/oval cells–mostly round/oval cells), 'abnormal calcification/debris' (present–absent), 'vascularisation within the repair tissue' (present–absent), 'mid-deep zone assessment' (bad–good) and 'surface/superficial assessment' (bad–good) (Fig. 3) (Table IV). A 14th category ('inflammation') may be included when a scaffold has been used during the cartilage repair procedure, as for example during matrix-assisted chondrocyte implantation. Similar to most other cartilage repair scores, this score does not evaluate integration of the repair tissue with its surroundings, which is due to the often limited size of a biopsy in the clinical setting.

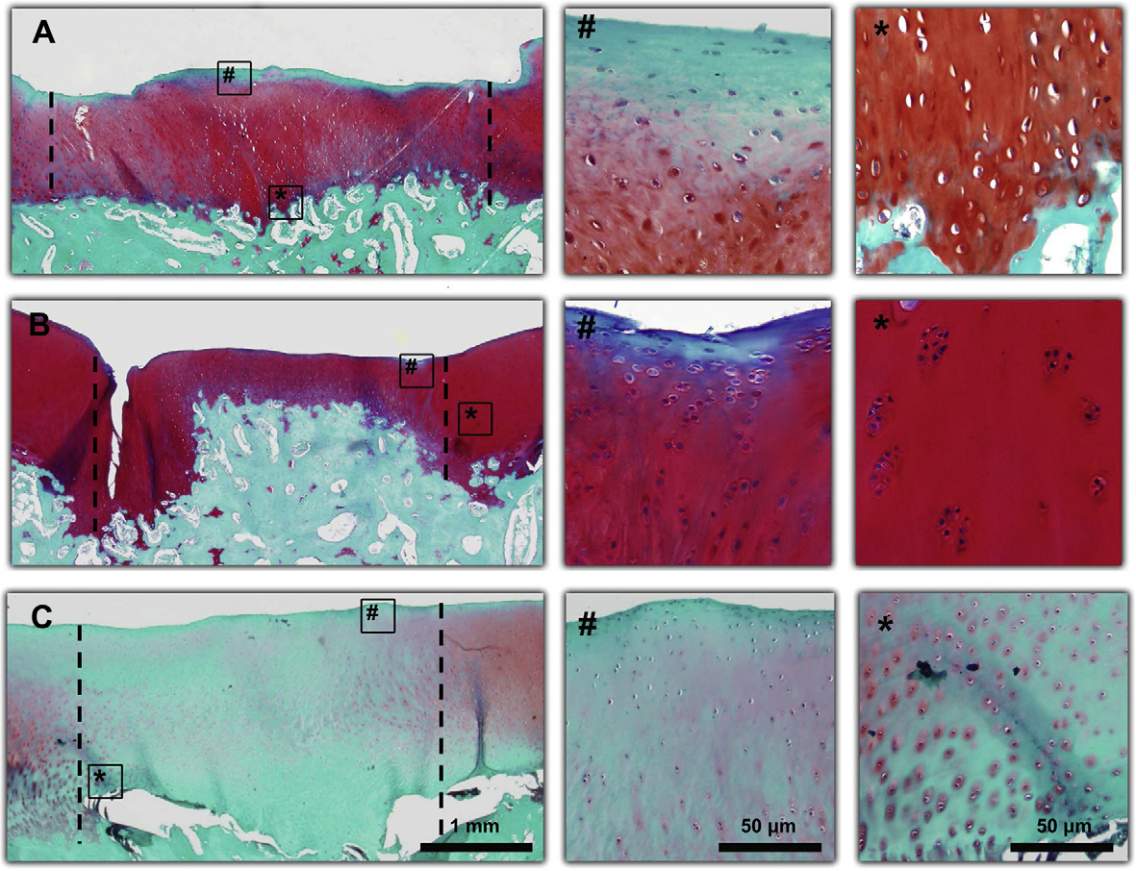

Fig. 3. Safranin O/Fast Green-stained histological sections of adult female Dutch Milk goat knee articular cartilage, after a microfracture procedure (5 μm sections). Sections were scored with the O'Driscoll score[6] and the ICRS II score[48] in small (left) and larger magnification (right images, # and *). ICRS II score categories are abbreviated as follows: overall; staining; morphology; clusters; tidemark; subchondral bone; surface architecture; basal integration; cells; abnormal calcification; vascularisation; mid deep zone; surface assessment. The microfractured area is between the dotted lines. (A) High quality repaired cartilage; assessment of 'bonding of the repaired cartilage with its surroundings' is possible with the O'Driscoll score, while the ICRS II score gives more detailed information about specific repair features as for example basal integration. (B) Moderate quality repaired cartilage; the ICRS II score acknowledges the more intense overall Safranin-O staining in this section [compared to the previous 'high quality' section, Fig. 3(A)] while the O'Driscoll score only provides a lower total score. Note the hypercellularity in the microfractured part of the tissue (#) in comparison to the cellular cloning in the non-microfractured part (*). (C) Low quality repaired cartilage; weak Safranin-O staining (#) and an absent tidemark are reflected in the ICRS II score, while these features are not discussed in the O'Driscoll score. While the overall O'Driscoll score of this section hardly differs from that of the previous section [Fig. 3(B)], significant differences exist between the ICRS II subcategories of these sections. Note the transition from Safranin-O stained tissue in the original cartilage to non-stained tissue in the repaired cartilage (upper left of magnification *), and the poor binding of the cartilage to the subchondral bone in the microfractured part (lower right of magnification *) compared to the non-repaired cartilage.

When comparing the O'Driscoll score and the ICRS II score in Fig. 3, differences in 'intensity of matrix staining' are not reflected in O'Driscoll subscores, while the ICRS II score does distinguish the more intense Safranin-O staining in the 'moderate' quality tissue, even though the 'good quality' cartilage has a higher O'Driscoll score [Fig. 3(A, B)]. Further, little difference exists between total O'Driscoll scores for 'moderate' and 'low' quality repaired tissue [Fig. 3(B, C)], while the ICRS II subscores are obviously different (i.e., staining and basal integration).

Tissue-engineered cartilage

As in OA and *in vivo* repaired cartilage, the quality of tissue-engineered cartilage is described using macroscopic morphological outcome parameters, immunohistochemical staining, quantitative histomorphometry, analysis of overall cellularity, DNA content, glycosaminoglycan content and collagen content[23,51–56]. Few histological scoring systems are available for the evaluation of tissue-engineered cartilage.

In 2001, O'Driscoll correlated a simple four-category subjective scoring system to an automated histomorphometry program determining the intensity of Safranin-O staining[9]. The score, in essence a modification of the Mankin score, showed a good correlation with the automated system and the authors suggest that this system is a good alternative to a computerized system. However, this automated system only focused on proteoglycan 'density', and did not evaluate characteristics as, for example, cell morphology.

Another grading system developed exclusively for the evaluation of *in vitro* engineered cartilage was presented in 2006[7]. This 'Bern score' showed a good correlation of cartilage quality with glycosaminoglycan content as measured biochemically and by automated histomorphometry.

Table III
Most cartilage repair scores (other than the Pineda, Oswestry, Knutsen and ICRS scores) are based on modifications of the O'Driscoll score or HHGS

Year	Authors	Comment
'95–'08	Several[63,84–97]	O'Driscoll category modifications; most important new or altered categories: 'reconstitution of subchondral bone', 'tidemark formation', 'basal integration of the graft' and 'signs of inflammation'
'97–'07	Several[66,98–100]	Includes a score which evaluates focal chondral defect treatment using a score based on HHGS[66]. As the O'Driscoll score, this score evaluates cartilage repair integration. Further, macroscopical evaluation is used in combination with histological evaluation
'01	Mainil-Varlet *et al.*[90]	O'Driscoll category modifications; 'chondrocyte clustering' is considered as a feature of repair tissue and not as a degenerative feature
'08	Nettles *et al.*[63]	Combination of O'Driscoll score and ICRS I (Visual Histological Assessment Scale). Although various 'new' scales combine features of earlier scales, this is one of the only systems that explicitly combines 2 scales

The Bern score was validated for the assessment of pellet-cultured cartilage, and has been used several times thus far[57–61]. Im *et al.*[62] modified the Bern score by addition of 'immunohistochemical staining for type II collagen' to the first scoring parameter. The influence of this modification on the validity of the original score was not evaluated. An advantage of the Bern score, is that it covers a broader score range and thus gives more detailed information on tissue characteristics, than for example the O'Driscoll score (Fig. 4).

Discussion

Evaluation of cartilage quality through histological analysis significantly contributes to the assessment of the extent of cartilage damage or the success of cartilage regeneration[1–3]. The development of *in vitro* tissue engineering techniques further extends knowledge of cartilage structure and composition. When evaluating cartilage characteristics, choosing the appropriate histological scoring system is important for analysis of cartilage pathology, evaluation of the result of its *in vivo* treatment or for further *in vitro* tissue engineering research. For each of these categories, different histological scoring systems exist.

Healthy cartilage is most often distinguished from osteoarthritic cartilage using the HHGS[5] or a HHGS-related score, even though reproducibility and validity of this score have been disputed[10,11]. Nevertheless, in our experience it is an adequate system for analysis of degenerative cartilage characteristics due to its overall comprehensive character. The widespread use of this system further facilitates comparison of one's histological data with that of other cartilage researchers. The many modifications of the HHGS necessitate the use of adequate terminology; 'HHGS' or 'Mankin score' for the original 1971 score, and 'modified Mankin' or 'modified HHGS' for scores in which a modification has been made, even if only one category is omitted.

An alternative for the HHGS is the OARSI system[12], which seems easier in use for inexperienced scorers[12,18] but has been used infrequently thus far. When the entire articular surface is included in a tissue section, as for example in joints of small animals, the influence of surface extent or 'stage' in the eventual score provides additional information. As the OARSI system seems more robust and the reliability is higher than the HHGS[18], this system will be preferable in most cases.

Of the various scores available for analysis of *in vivo* repaired cartilage, the ICRS II score[49] seems most suitable,

Table IV
Parameters assessed by some major cartilage repair scores

Parameters of the different grading systems	O'Driscoll *et al.*[6]	Pineda *et al.*[36]	Wakitani *et al.*[37]	Modified Mankin (Uhl *et al.*)[66]	OsScore[20]	ICRS I (VHS)[8]	Knutsen *et al.*[2]	ICRS II (VAS)[48]
Cell morphology	×	×	×	×	×	×	×	×
Matrix staining	×	×	×		×			×
Surface regularity	×		×	×	×	×		×
Structural integrity	×							×
Thickness/defect filling	×	×	×					
Osteochondral junction	×	×						×
Adjacent bonding	×		×	×				
Basal integration					×			×
Cellularity	×							
Clustering/distribution	×				×	×		×
Adjacent cartilage degeneration	×							
Mineral					×	×		×
Blood vessels				×	×			×
Subchondral bone						×		×
Viability cell population				×		×		
Inflammation								×
Cartilage plug quality				×				

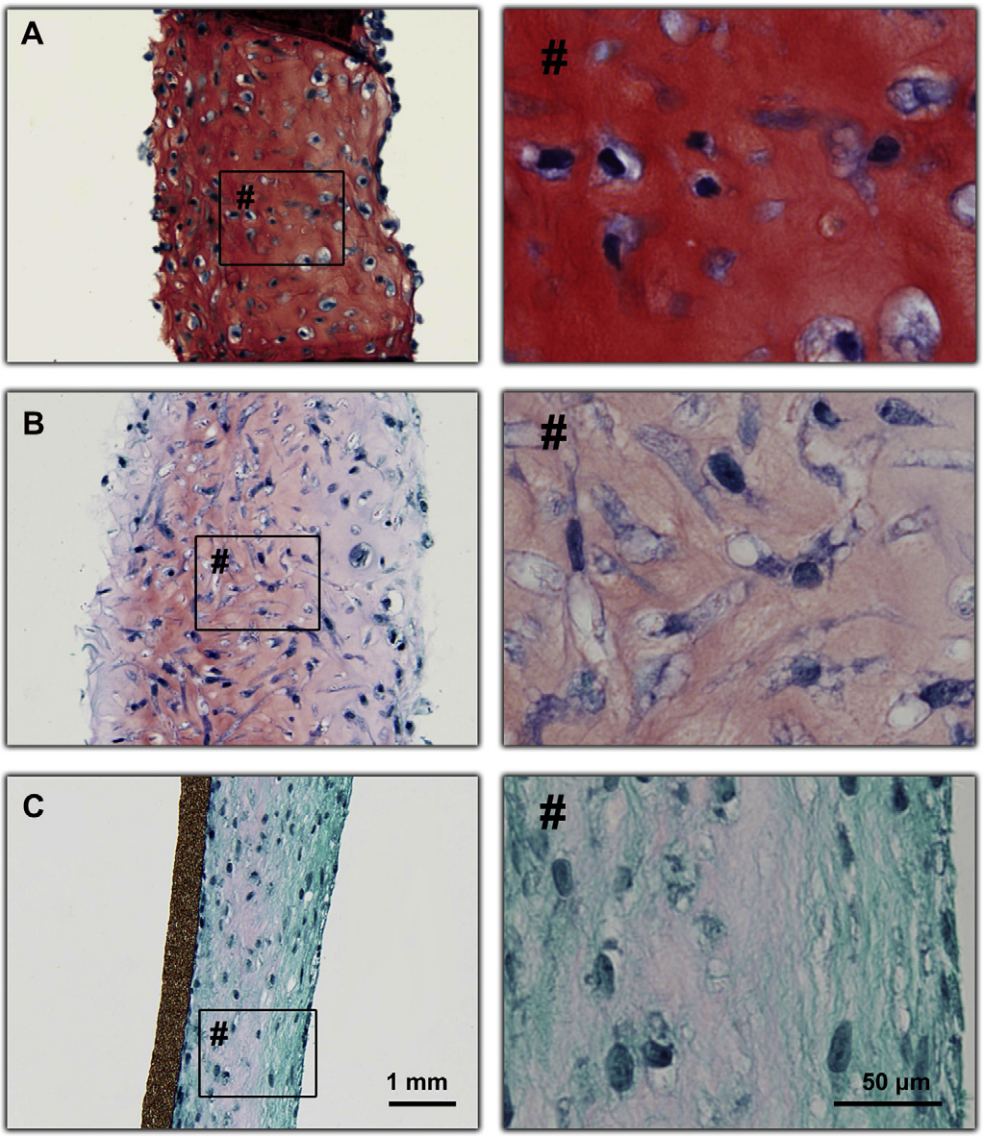

Fig. 4. Safranin O/Fast Green-stained histological sections of tissue-engineered (TE) cartilage (healthy human knee articular chondrocytes, passage 2, cultured for 28 days in medium containing transforming growth factor β2, 5 μm sections). Sections are scored using the Bern score[7] and the O'Driscoll score[9]. (A) TE cartilage of high quality; note the intense Safranin-O staining and high amount of matrix as well as chondrocyte-like cells in the tissue. (B) TE cartilage of moderate quality; note the less intense Safranin-O staining and the combination of fibroblast-like cells and chondrocytes, resulting in a lower Bern score. (C) TE cartilage of low quality; a thin matrix and hardly any Safranin-O staining leads to an even lower Bern score. The Bern score obviously facilitates more subtle distinction of slight changes in tissue quality through its broader range (Bern score: 3–9 vs O'Driscoll: 0–3).

as it is validated, comprehensive and describes each cartilage characteristic individually. Further, this score proved valuable during analysis of biopsies in the course of a recent randomized controlled clinical trial[3]. As biopsies of human cartilage are often small and do not include a border region between repaired cartilage and its surroundings, one may choose to comment on this separate from the score. Alternatively, the O'Driscoll score[6] may be used.

For evaluation of *in vitro* engineered cartilage, the 'Bern score'[7] is preferred as it is validated and adequately distinguishes between characteristics specific for tissue-engineered cartilage[57–61].

Sporadically, authors use two different histological scoring systems in a parallel fashion, or combine the scoring systems into one, in essence creating a new scoring system[63]. The difficulty of combining scoring systems is that the mutual parameters of each scoring system (for example: proteoglycan staining) are over-emphasized in the eventual score, thus decreasing the 'impact' of separate categories of the individual scores. Using scoring systems in a parallel fashion results in more information on the histological characteristics of the assessed tissue, however using one comprehensive validated system is preferable over using several non-validated systems.

Although validation is one of the major motivations for the application of a particular scoring method, the ideal validation method remains disputable. Ideally, a histological score is validated by comparison to several parameters important to cartilage, i.e., by comparison to (validated) biochemical, biomechanical, macroscopical and (functional) imaging

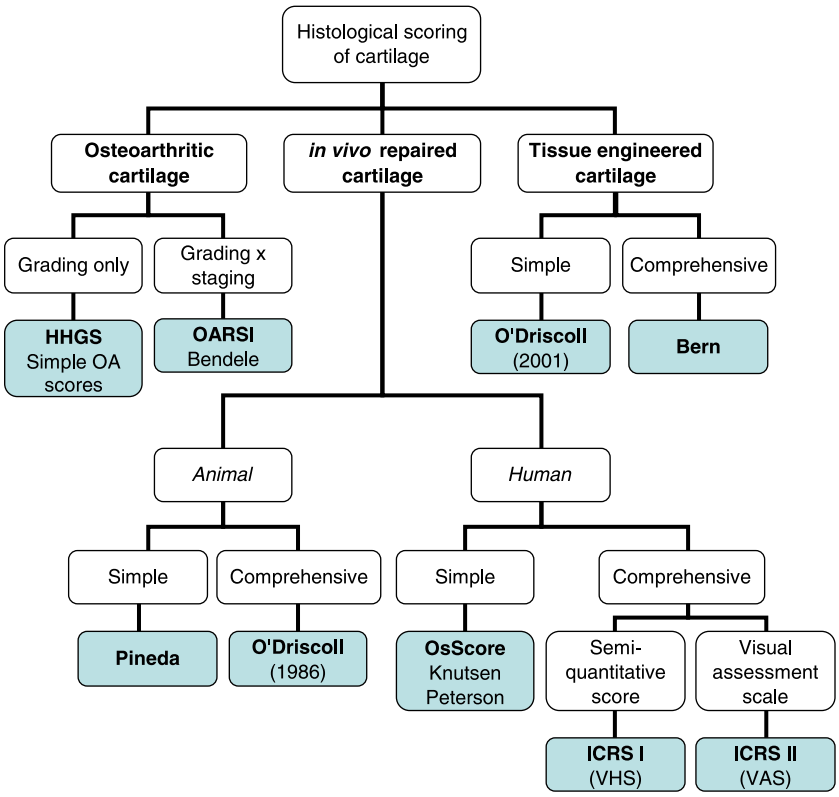

Fig. 5. Choice of an appropriate scoring system may be aided by a flow diagram. Validated scores are presented in bold.

techniques. Of these parameters, regardless of the tissue type (OA, repaired or tissue-engineered cartilage), correlation to bio*chemical* parameters seems important. Nevertheless, bio*mechanical* properties are important for repaired cartilage as well and biomechanical testing techniques as for example 'indentation stiffness' may add to the qualitative description of the tissue[64]. However, these techniques may have their limitations (i.e., analysis of cartilage repair after microfracture using indentation stiffness is limited due to outgrowth of subchondral bone)[65]. The ideal combination of analysis techniques therefore remains to be developed. In the future, not only uniformity in the application of histological scores, but also in the use of validation techniques will aid development of improved cartilage analysis techniques. Even when adequately validated, additive information on macroscopical, biochemical and biomechanical aspects of the tissue will yield a more complete picture of tissue quality[64,66].

In conclusion, a variety of histological scoring systems exists for analysis of osteoarthritic or normal, *in vivo* repaired or *in vitro* tissue-engineered cartilage, but only few have been validated. For each category, specific validated histological scores emerge as most suitable for application. Use of these validated scores may considerably improve exchange of information necessary for advances in the field of cartilage regeneration and thus stimulate uniformity enabling comparison of results of different cartilage research groups (Fig. 5).

Conflict of interest

The authors declare that they have no proprietary, financial, professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of this manuscript.

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