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Osteoarthritis and Cartilage

Letter to the Editor

Response to Letter to the Editor: 'India ink and cartilage'

We would like to thank Dr Aspden for his insightful comments on Indian ink and we totally agree that there is confusion with respect to this terminology in the literature. Indeed his letter provides us with an occasion to expand on this particular subject and make some suggestions as to how to improve and standardize these methods in the future (one of the goals of the OARSI histopathology initiative). First of all Indian Ink is the term employed in Britain and India Ink is more commonly used in North America. Part of the confusion with respect to the use of Indian ink for cartilage research arises due to the lack of specific information in many early publications, cited by Dr Aspden, as to the brand/source of Indian ink employed or how it was prepared. This also includes two publications on which Dr Aspden is an author[1,2]. Furthermore, there is no single Indian ink or fixed formula and the composition is rarely revealed by the manufacturers. In addition, some claim that Indian ink was probably invented in China, but employing carbon from India, and this makes the tale even more confusing. Indian ink is called "encre de Chine" or Chinese ink in French! It would appear, that the Indian ink that is most similar to the publications earlier this century, cited by Dr Aspden, would be Indian inks employed for calligraphy (fabricated from pine soot) that needs to be ground down with an inkstone and diluted with liquid.

In his seminal studies Meachim[3,4] reported the method for detection of fibrillation of human articular cartilage employing Indian ink but, unfortunately, did not provide details on the source or preparation of the Indian ink employed to allow others to repeat exactly what he did. Chang[5] was one of the first to actually provide the source of the ink that they employed to highlight fibrillation in a rabbit model of osteoarthritis. They used Design Higgins waterproof drawing ink, black India 4415, Eberhard Faber, Lewisburg, TN, USA and indicated that it contained 6% carbon black particles (information provided by the ink manufacturer on request)[5]. However companies that fabricate ink are usually not very willing to divulge the composition of their ink, so it is difficult to determine the exact amount of carbon particles in the ink. Rarely the properties of ink have been researched[6].

In our article we provided a reference for a recent publication employing ink staining[7]. One of us also employs Sheaffer's Ink Jet Black (Fig. 1) for the purpose of highlighting fibrillation in a rabbit model of osteoarthritis[8]. When employing the latter ink, the authors have observed a similar pattern to that reported by Meachim when employing Indian ink, namely: "Sites showing dark markings against a pale grey background, termed 'minimal fibrillation'; …Sites showing semi-confluent or confluent blackening, usually indicating 'overt fibrillation' of the cartilage"[3]. The latter has satisfactorily highlighted (enhanced visualization of) fibrillated areas for the purposes of macroscopic scoring. Another of us routinely and successfully employs 10% diluted Higgins waterproof drawing Black India Ink (4415). So it appears that there are many roads to Rome.

In response to Dr Aspden's comment "Whether these inks are superior to more conventional stains for cartilage histology, I suggest, needs to be proven" (sic). We never stated that these stains were "superior" to other methods and did not refer to histology. We recommended them solely to enhance visualization of fibrillation for the purposes of macroscopic scoring of cartilage lesions. It would be interesting to compare the calligraphy Indian/Chinese inks to the other inks to establish whether they are indeed superior for macroscopic scoring of articular cartilage fibrillation. The authors suspect that the contrast between fibrillated and normal cartilage may be better with the former.

We agree that solvents in inks could be a problem if additional analyses were to be performed on cartilage and this was pointed out clearly in both the article on *Basic methods in histopathology of joint tissues*[9] and repeated again in the article The OARSI histopathology initiative recommendations for histological assessments of osteoarthritis in the rabbit[8] dealing with the macroscopic evaluation of rabbit cartilage.

"Although Indian ink itself is inert, its solvent could potentially interfere with certain analyses (e.g., immunohistochemistry, molecular biology) it would therefore be prudent to avoid its use when additional analyses are required or tested to insure compatibility with the particular analytic process"(sic).

As we mentioned in the opening chapter of this initiative *The OARSI histopathology initiative – the tasks and limitations*, this work will need to develop and improve. It is only the beginning and not a final consensus. All scoring systems proposed will require further validation.

In conclusion we propose that, ideally a study should be performed comparing different Indian inks and writing inks now available on the market to determine which are the optimum for detecting articular cartilage fibrillation. Ease of use and availability should be taken into consideration. Also we would like to propose that, in all future studies employing inks to highlight cartilage fibrillation, information should be provided on the ink employed and it's source in an attempt to harmonize and standardize methods across laboratories.

Conflict of interest
None.

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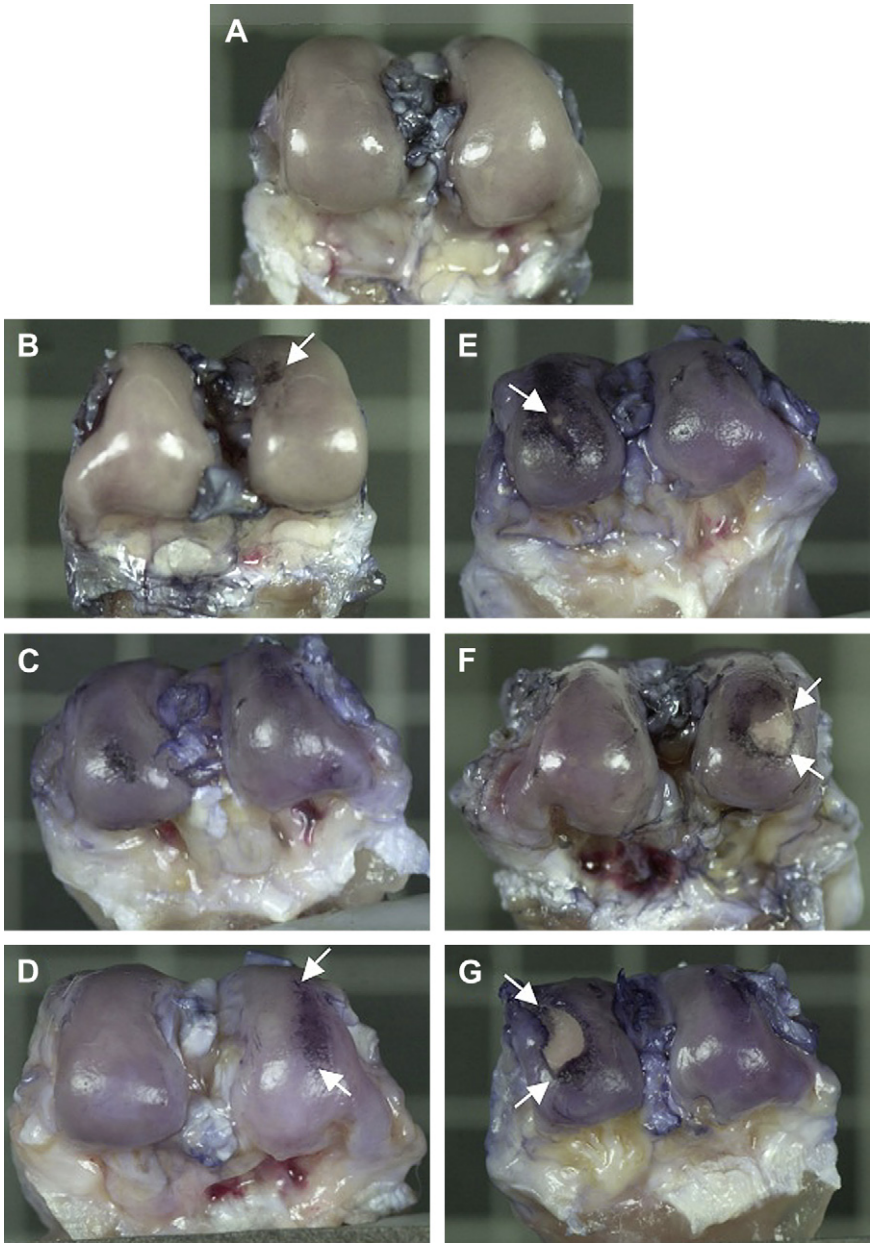

Fig. 1. Representative macroscopic changes of the articular cartilage. Ink particles are absorbed to the cartilage surface or trapped in clefts and indicate areas of fibrillation. With cartilage ulceration, the bone appears as a pale area surrounded by ink stained cartilage, highlighting fibrillation in cartilage contiguous to the ulceration.
(A) **Score 1**: Intact surface: surface normal in appearance and does not retain India ink
(B) **Score 2**: 0 mm < Fibrillation ≤ 4 mm
(C) **Score 3**: 4 mm < Fibrillation ≤ 8 mm
(D) **Score 4**: 8 mm ≤ Fibrillation
(E) **Score 5**: 0 mm < Ulceration ≤ 2 mm
(F) **Score 6**: 2 mm < Ulceration ≤ 5 mm
(G) **Score 7**: 5 mm < Ulceration.

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N. Schmitz, S. Laverty, V.B. Kraus, T. Aigner*
University of Leipzig, Institute of Pathology, Liebigstrabe 26, 04103 Leipzig, Germany

* Address correspondence and reprint requests to: T. Aigner, Medical Center Coburg, Institute of Pathology, Ketschendorferstr. 33, 96450 Coburg, Germany. Tel: 49-9561-226213; Fax: 49-9561-226219.
E-mail address: thomas.aigner@klinikum-coburg.de (T. Aigner)