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REVIEW

Auto fluorescence of intervertebral disc tissue: a new diagnostic tool

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Abstract The paper reports on auto fluorescence phenomena of inter-vertebral human discs. It systematically investigates the auto fluorescence effects of ex vivo disc specimen and reports on surgical cases to demonstrate the potential value of the new method. The paper offers biologic explanations of the phenomenon and discusses the potential value of the UV auto fluorescence technique as a diagnostic tool. Intra- and postoperative observations are made by a surgical microscope with an integrated UV light source. Quantitative measurements were carried out using a photon counter and a spectrometer ex vivo. The auto fluorescence phenomenon allows the differentiation of traumatized and degenerated disc tissue intraoperatively in some cases, it allows the differentiation of bony and collagen endplate in cervical disc surgery. The source of the auto fluorescent light emission are amino acids of the collagen molecules. The proteoglycan components and the liquid components of the disc do not show relevant auto fluorescence. Emission wavelength of disc material is equivalent to color perception. It differs due to different collagen composition of the intervertebral disc components from yellow-green to blue-green and can be visualized in situ by naked eye.UV-auto fluorescence of inter-vertebral discs is a new clinical tool that has the potential to differentiate disc material from the anatomical surrounding, to distinguish between different fractions of the disc and to give information on the quality and status of the disc material. Since the technology has just emerged, it needs further investigations to quantify the clinical observations reported in this paper.

Keywords Auto fluorescence · Intervertebral disc · Collagen · Human disc tissue

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Introduction

The auto fluorescence phenomenon of inter-vertebral disc tissue was known since 1964 [18]. However, no clinical study has systematically investigated the clinical value of this phenomenon. We offer the first systematic investigation on auto fluorescence of the different anatomical fractions of the disc and report how traumatized disc tissue or tumors alter the auto-fluorescence emission.

The origin of auto fluorescence

The auto fluorescence of collagen, elastin and other fibers in human tissue was observed and described first

at the time of the invention of strong UV light sources at the beginning of the last century [23, 28, 30]. The auto fluorescence of disc tissue is triggered by UV light with a frequency between 390 and 415 nm with a peak at 410 nm. The molecular source of the phenomenon are specific amino acids of the collagen molecules [11]. Keratin and Elastin molecules of connective tissue emit auto fluorescent light at 430 nm. Both molecules are rare in intervertebral discs and do not contribute to disc auto florescence [2, 5, 8, 9]

All collagen molecules in all tissues emit auto fluorescent light under UV irradiation [7, 13, 20, 25]. Other molecules—particularly hemoglobin—extinct the auto fluorescence [12]. A strong absorption band of hemoglobin, the so-called Soret band is responsible for the extinction, Fig. 1.

However, the intervertebral disc is a vessel and blood free compartment. This allows immediate intra- and postoperative perception of disc auto fluorescence whereas the auto fluorescence of muscle or mucous tissue is barely visible and barely measurable. Despite the difficulty in quantification and qualification of auto fluorescence of other tissues than the intervertebral disc, the measurement of native auto fluorescence is used for the diagnosis of tumors. Superficial tumors in the human airways, the intestine or the bladder display specific auto fluorescent patterns [7, 13, 19].

The paper deals with the auto fluorescence effect of disc material that can be visualized with the surgical microscope e.g. in cervical surgery. It enlightens the quantitative and qualitative effects of different disc fractions and their relation with the histological compositions. We further report on quantitative spectroscopy of auto fluorescence in ex vivo preparations.

Methods

To give a comprehensive description of the phenomenon of disc auto fluorescence, intra-operative observations and cases were reported. Systematic investigations and quantitative measurements were carried out on ex vivo disc specimen after surgery.

Methods of the intra-operative observation

A Moeller-Wedel surgical microscope (Moeller-Wedel-Haag Streit, Wedel, Germany) was prepared with a Wolf light source (Richard Wolf, Knittlingen, Germany). The light source emitted white light to support the surgical procedure and UV/blue light to investigate the auto fluorescence of the disc. UV/blue light was transferred via a liquid filled light cable since the normal glass fiber cable absorbed the UV light quickly. The emission wave-length of the UV light source had a peak at 415 nm (Fig. 2). It was possible to switch between white and UV/blue light. The light source was originally built to support glioma surgery with the help of aminolaevulinic acid (ALA).

Patients and realization

Intraoperative investigations with UV/blue light were performed over a 1-year period in 42 cervical surgeries,

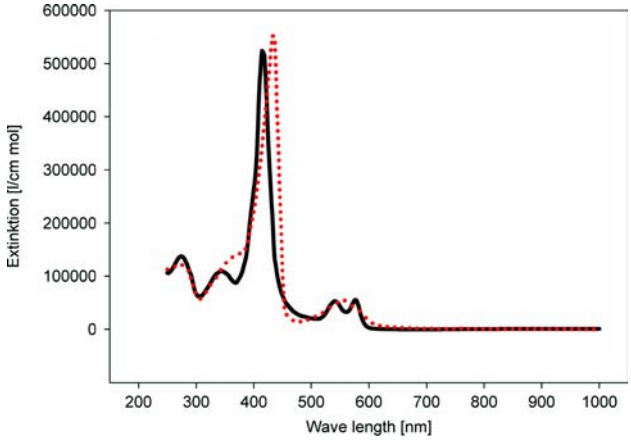

Fig. 1 Both graphs display the absorption of oxygenated (*solid black line*) and deoxygenated (*dotted red line*) hemoglobin. The absorption peak of hemoglobin at about 410 nm is in the same range as that of the maximum absorption of collagen molecules. This explains the extensive extinction of auto fluorescence in the presence of blood contamination

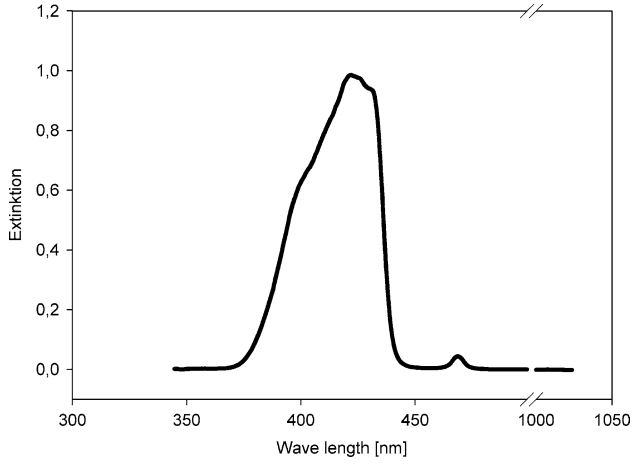

Fig. 2 The figure displays the measured spectrum of the UV light source (Richard Wolf, Knittlingen, Germany) that was used in combination with the surgical microscope (Moeller-Wedel-Haag Streit, Wedel, Germany). The broad peak and the high intensity of the light source allowed the excitation of all auto fluorescent molecules of the disc tissue

20 lumbar and 4 posterior lumbar inter-body-fusions (PLIF). Surgery was performed with white light. After surgical preparation of the situs, it was rinsed with saline solution to remove the blood. An electronic switch at the microscope drove a filter box in front of the light source to extinct all light except the ultraviolet/blue part. The situs turned black and only the auto fluorescent tissue could be seen in green.

It was noted whether auto fluorescence was observable or not. It was further noted if the anatomical structures of the disc were displayed more differently as with white light.

Methods of the ex vivo investigations

Qualitative and quantitative measurements of absorption and emission were carried out ex vivo under lab-conditions. These postoperative investigations of disc material helped to overcome the shortcomings of the human eye in the observation of auto fluorescence. Disc specimen of 64 lumbar disc patients were investigated and photo documented directly after surgery. The 300 W Xenon light source form Wolf, Germany was used as UV illumination technique.

The intensity of auto fluorescence was measured on a semi-quantitative scale that consisted of auto fluorescent synthetic colors. Color intensity and color quality were noted. Intensity was graded on a scale. No auto fluorescence was graded ''0''. Maximum auto fluorescence compared to the synthetic color was graded ''5''.

To correlate the fluorescence with the underlying structure of the collagen matrix, every specimen was investigated histologically. The vital chondrocytes were counted per visual field to clear up the relationship between number of cells, and the intensity of auto fluorescence. The hypothesis was that collagen matrix production of vital chondrocytes was correlated with the auto-fluorescence intensity [14, 26]. Single specimen were measured with a photon counter (LMZ, Lübeck, Germany) and a spectrometer (MUT, Wedel, Germay) to obtain spectra.

Results

Systematic intra-operative observation in disc surgery

The results of the intra-operative investigation are presented in Table 1. It was clearly shown that the technique worked well with ventral cervical surgery. The endplates could be clearly identified in nearly every case. In two cases auto fluorescence was not observable due to ongoing hemorrhage in combination with surgical difficulties. In 35 of 42 cases, the differentiation between endplate and nucleus and bone was easy to make under UV/blue light.

Table 1 Results of the intra operative auto fluorescence investigation. Values given in *n* number. *PLIF* posterior lumbar inter-body-fusion

Surgery	Investigation possible		Structures differentiated	
	Yes	No	Yes	No
Cervical disc (n_{total} = 42)	40	2	35	5
Lumbar disc (n_{total} = 20)	12	8	2	10
PLIF (n_{total} = 4)	2	2	1	1

In cases of significantly degenerated disc structures, the disc structures were chanced to solid unstructured material of uniform collagen. Those degenerated cases emitted only a little blue/green light. It remained unclear if the degeneration of collagen molecules of the disc reduced emission or if the material was contaminated (Fig. 3).

Results of the auto fluorescence investigation of lumbar discs

In lumbar disc surgery and in PLIF surgery, the auto fluorescence technique did not work in two-thirds of the cases (Table 1). This indicates that the technique at the present state of development may not add value to surgical procedures. The main reason for the poor results was significant blood contamination in lumbar cases. Other reasons were the auto fluorescence of the dura and ligaments. The auto fluorescence of these structures was noticeably weaker than that of blood free disc material but interfered with the low signal of the contaminated disc material.

Auto fluorescence of bone and tumor

In all cervical and lumbar cases, bone did not show any visible auto fluorescence during standard disc surgery although it consists of comparable collagens. The reason was the ubiquitous presence of blood between the bone trabecula that led to complete extinction of the auto fluorescence. If bone was rinsed intensely under high pressure with saline solution and all erythrocytes and hemoglobin material were removed, it displayed a visible auto fluorescence. This procedure was per-

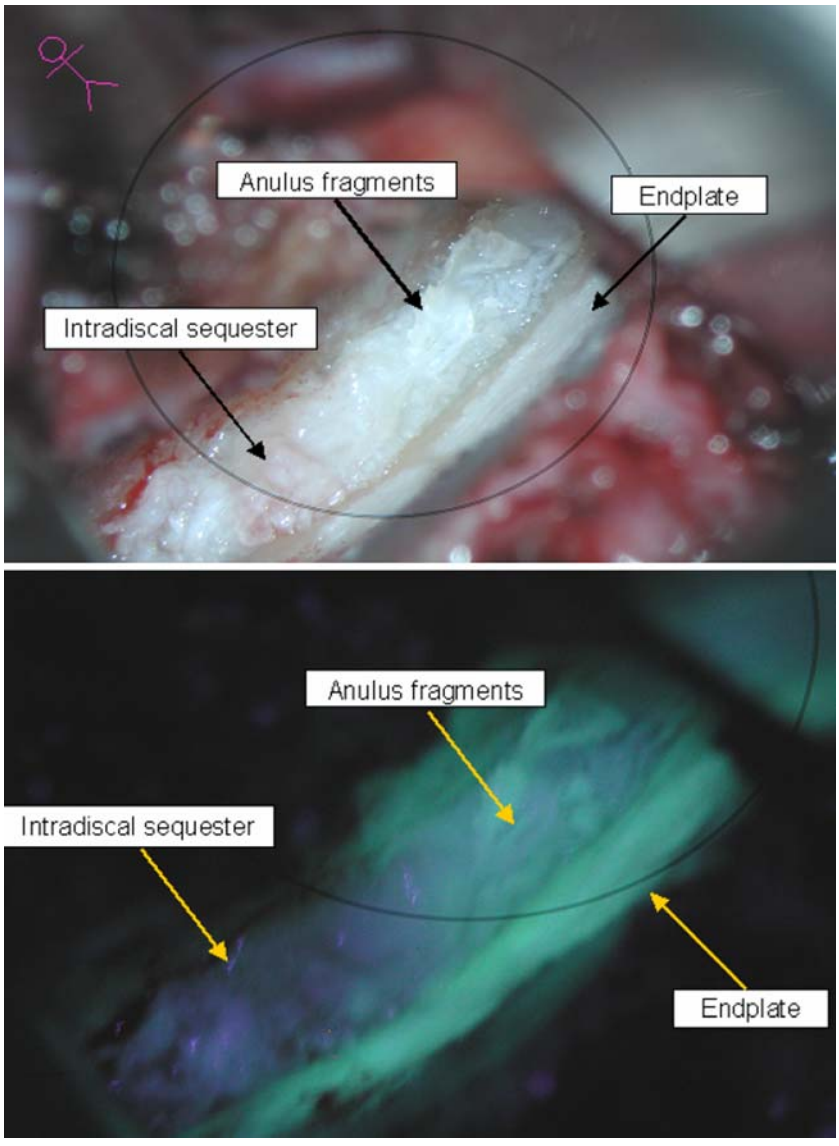

Fig. 3 The *upper part* of the image shows in white light the intraoperative situs in a ventral cervical approach. The nucleus was already removed. All parts of the disc showed different shades of *white colors*. The sequester was slightly *reddish* due to a minor blood contamination. The *lower part* displays the auto fluorescence situation. The endplate was *bright green*. The auto fluorescence of the sequester was significantly reduced due to the extinction of auto fluorescence by the hemoglobin contamination of the sequester

formed in two cases of cervical mama tumor metastases in bones. The tumors extincted the auto fluorescence of the collagen material of the cervical vertebrae. The extinction zone was larger than the visible tumor infiltration zone at white light observation. Histology of the tumor infiltration zone revealed a good correlation with the UV/blue light appearance of the tumor. The true tumor infiltration zone could not be located precisely in white light, (Fig. 4).

Auto fluorescence of traumatic disc prolapse

We investigated three cases of traumatic disc prolapses. General observation was the extinction of auto fluorescence in sequestered material. The extinction was demonstrated in three cases of high-speed car accidents and consecutive large disc prolapse. Figure 5 demonstrates the postoperative specimen. The auto fluorescence behavior and the MR image are demonstrated. The main difference between traumatic sequesters and those of patients with a degenerative history of disc is the complete extinction of the auto fluorescence in the traumatic sequester. The extinction was complete in one case even after 4 weeks post trauma.

Results of the ex vivo investigations

One obvious result was that the different anatomical parts of a lumbar disc reveal distinct auto fluorescence, Fig. 6a and b. Histological investigation of the different fragments showed that the bright ones were from the endplate and the dark ones were either contaminated with blood or consisted of nucleus material. To

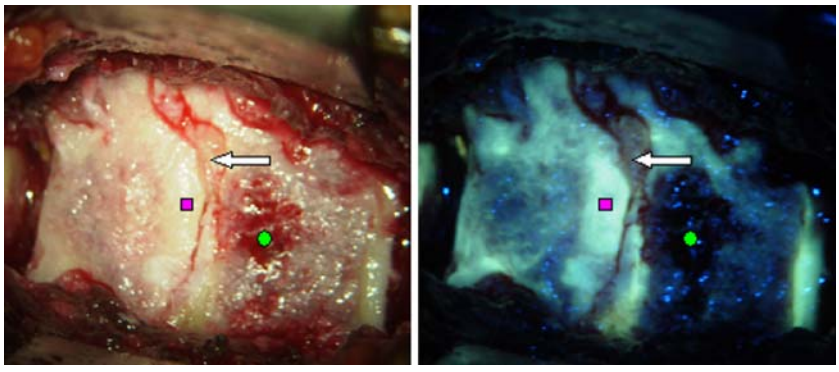

Fig. 4 Cervical situs from ventral. On the *left side* illuminated with white light, on the *right side* with UV light. The bony surface was milled away. The *white arrow* indicated the blood contaminated disc surface. The *green dot* marks the tumor. The true size of the tumor is obviously larger than it seemed with white light. The *pink dot* indicates the bony endplate of the vertebra. UV light may help in those cases to better identify the infiltration zone of the tumor

understand the relationship of auto fluorescence and its underlying nature, quantitative investigations of auto fluorescence were carried out.

The semi quantitative measurement of auto fluorescent brightness and cell count displayed a strong relationship. The endplate was in all specimen the

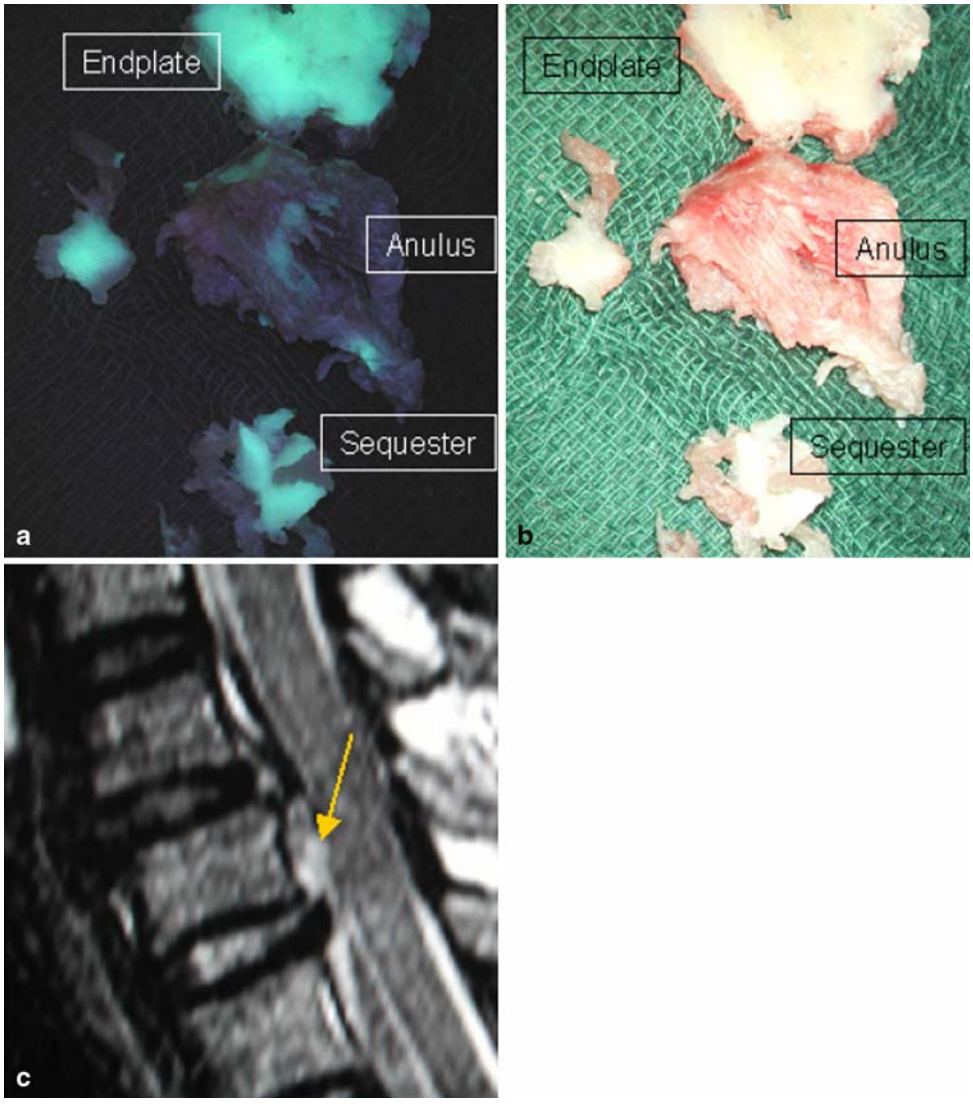

Fig. 5 **a** Different disc fractions directly postoperative in UV/blue light. The Sequester has lost its auto fluorescence due to hemoglobin contamination. **b** Shows the same ensemble in white light. The sequester appears reddish despite saline rinsing. Histology of the sequester could not reveal erythrocytes though intermingled hemoglobin might well be the reason for the change in auto fluorescent behavior. **c** T2 weighted image with the cranial sequestration and compression of the myelon (*arrow*)

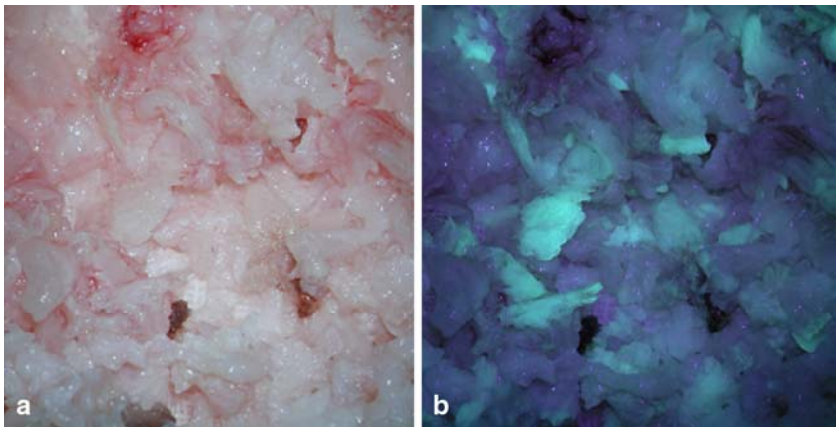

Fig. 6 **a** The white light image shows no relevant difference between the single fractions of the disc material. **b** displays the auto fluorescence image of the same specimen. Some fragments appear brighter than others. Histology revealed, that the bright particles came from the endplate and the anulus. The dark fragments were from the nucleus

probe with the brightest auto fluorescence had the highest cell count. The nucleus was the probe with the least cells and the least content of collagen displayed only a little auto fluorescence. Anular fragments differed over a wide range. Figure 7 shows the relationship.

The quantitative measurement of spectra came to comparable results with the semi-quantitative observation reported above. In all cases, the endplate displayed the highest quantitative value and the nucleus the lowest. The cases varied with age and grade of degeneration. Figure 8 shows a typical spectroscopic measurement and the histology of the same specimen. The relationship between the peak maxima of the spectroscopic measurement was about 1 (nucleus) to 1.5 (anulus) to 4 (endplate). This means that the endplate emits roughly four times as much photons as the nucleus.

Discussion

Discussion of the intraoperative cases

The results indicate that auto fluorescence may work well in cervical cases. Auto fluorescence helped to identify endplate remnants during cartilage endplate preparation in cases of cervical fusion.

In severe degenerated cases, auto fluorescence showed the disc remnant as a small green line between the vertebrae. The technique was helpful to mill the optimal approach along with the disc remnants.

The almost complete extinction of auto fluorescence in trauma cases was named a ''UV-black-sequester''. The disc material except the endplate did not light up in the presence of UV light even after intense washing with saline solution. Histology of those cases did not show any intact erythrocytes.

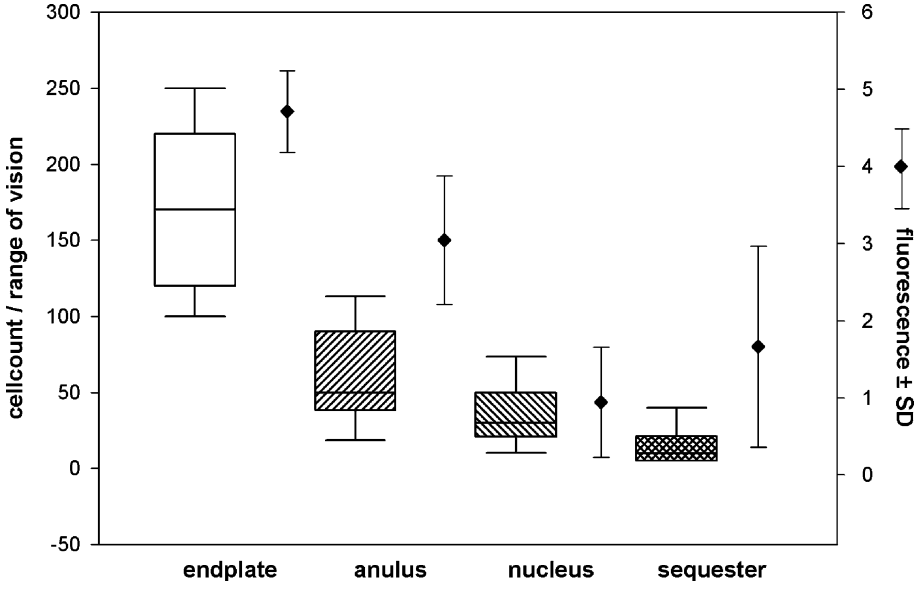

Fig. 7 The figure shows cell counts versus auto fluorescence brightness grouped due to their anatomical localization. *Left ordinate* cell count as box plot. *Right ordinate* brightness of auto fluorescence as mean value. The whiskers represent the 10th and 90th percentile, respectively

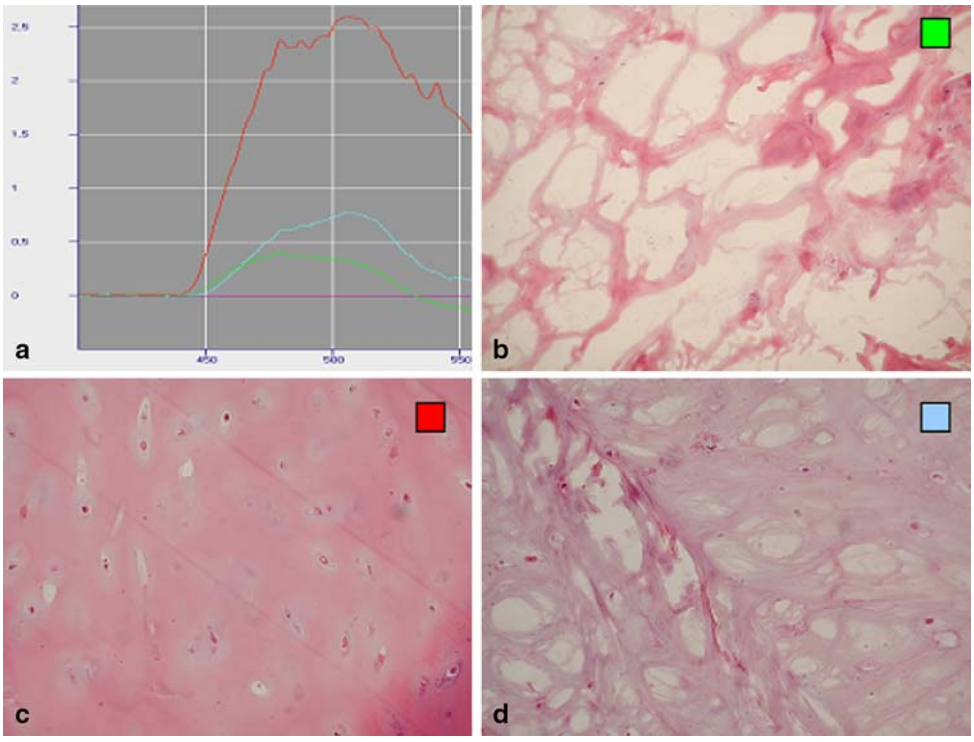

Fig. 8 **a** Spectroscopy of the fragments, *Y*-axis: photons counted in relative numbers; *X*-axis: wavelength in nm. *Red graph*: endplate, highest values, see the histology of the endplate at **c** indicated by a *red square*. The *blue graph* in **a** displays the measurement of the anular tissue, *blue square* at **d.** The *Green graph* shows the measurement of the nucleus with the lowest values (**b**)indicated by the *green square*

We offer the opinion that the traumatic event has opened the disc to the spinal canal or the bone endplate. It further had destroyed the blood corpuscles whereas the hemoglobin molecules were still existing to extinct the auto fluorescence. The observations indicate that the complete extinction of auto fluorescence is the sign of a recent trauma that was able to disrupt either the anulus or the endplate and open the otherwise blood free disc space. This observation is subject to further investigations since UV technique offers an intraoperative argument to distinguish between traumatic and nontraumatic origin of a cervical sequestration. The findings are true only in cervical cases since no traumatic cases in lumbar surgery have been investigated.

Discussion of lumbar intraoperative investigations

The observations in lumbar spine indicated that the technique had no clinical benefit in lumbar cases. The main reason was that blood contamination in lumbar disc surgery was found to be more frequent than in cervical disc surgery. The intraoperative investigation revealed that auto fluorescence technique was not needed in standard clinical procedures. In future UV application may be worthwhile in endoscopic lumbar procedures to distinguish between the spinal nerves and the sequester.

Discussion of the ex vivo investigations

The relationship between cell count, collagen content and auto fluorescence was investigated. Due to mathematical reasons a correlation analysis of both measurements was not admissible, but the result clearly shows that cell count and auto fluorescence correlate in the different anatomical fractions. One explanation of this result might be the fact that vital cells produce collagen in relative stable amounts per time over lifetime. This argument is supported by results of the ''German Disc Study''. The study shows that juvenile cases revealed more chondrocytes and brighter auto fluorescence whereas the aging disc had less cells, increased matrix proteolysis and an increased level of matrix degrading enzymes like metallo-proteases [21, 22]. Potential reasons of the age dependence of cell count and collagen content are for e.g. endplate degeneration with consecutive decreased levels of glucose and oxygen [14, 24].

Nucleus, anulus and endplate showed a strong relationship between cell count and brightness. The sequester did not. This observation remained the same in all measurements reported in this paper. The histological examination of the sequester offered a good explanation since the degradation of the sequester had reduced the number of vital cells in some specimens and had led to significant collagen destruction in oth-

ers. The effects were not systematic, they may depend on individual and patho-physiological differences or on the time between sequestration and surgery.

The quantitative spectral measurements showed that the peak differences in emission spectroscopy varied in about 20% of the cases up to 30 nm between the endplate and the nucleus. This means that different anatomical fractions show up different colors. Color differences were also seen between young and old patients. Young patients revealed blue-green color of endplate and anulus whereas patients beyond 70 years displayed yellow-green auto fluorescence. The systematic studies on quantitative spectroscopy are ongoing. Molecular degradation of collagens may be one, the formation of ageing pigments of different colors may be an other explanation [4, 6, 16].

The changes in color may well reflect the different composition of collagens or state of degeneration between the different probes [1, 3, 10, 15, 26, 27]. It is too early to correlate the color differences with morphological changes and clinical parameters. However, the quantitative measurements indicated that spectral analysis of the auto fluorescence might give information on the status of the disc.

Conclusion

Auto fluorescence of collagen molecules has long been known in basic science [17, 29, 31]. However, an evaluation of the auto fluorescent potential of the intra vertebral disc was not published yet. The paper offers a first clinical description of the phenomenon and indicates some potential applications.

The intraoperative use of the technique will be easy in the near future for those surgeons who use the new UV-ALA technique in Glioma surgery. Neurosurgeons who use the UV technique for brain tumors can use the same equipment to go on with auto fluorescence investigations of the disc. This will help in gaining a widespread data base and will be the foundation of a systematic investigation of the cases reported here.

We assume that the quantitative spectral emission analysis has the potential to add diagnostic value for the understanding of disc disease and disc degeneration in the future.

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