Application of In Situ Multi-Elemental Imaging With Laser Spectroscopy to Periprosthetic Tissue Characterization

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BACKGROUND

The elemental identification of metal particulates and solid corrosion products in periprosthetic tissues requires spectroscopic analysis such as elemental analysis of x-rays. Recently, an all-optical instrument based on laser-induced breakdown spectroscopy (LIBS) was developed to create multi-elemental images of biological tissues (1). The aim of this study was to examine the application of LIBS to the identification of metallic wear and corrosion products in standard paraffin blocks from a range of hip arthroplasty revision cases.

METHODS

Archived paraffin blocks from 9 revised total hips and one primary tissue were used. The general location of microscopic debris in the H&E slides was noted on a slide grid map (Figure 1) and the histology features were ranked with a 0 to 3+ semi-quantitative score (Figure 2, Table 1). For LIBS (Figure 3), the wax blocks were examined directly in ambient conditions using a Nd:YAG laser operating at 1064 nm, pulsed at 100 Hz, pulse energy of 4 mJ/ shot. Each part of the tissue was scanned, pixel by pixel, with a resolution of 50 µm. The typical spectral range used was from 190 to 362 nm. The signal intensity for elements of interest (eg. Co, Cr, Ti, Fe) were converted to a map image by a false color scale. These maps were compared with the histological features of the H&E sections in corresponding sites.

Feature per 40x high power field (2)	1+	2+	3+
Mononucear Histiocytes	1-5	6-49	50 or more
Lymphocytes	1-9	10-49	50 or more
Giant Cells	1	2-4	5 or more
Metal Particles	Slate Blue cells	Dusty black cells	Jet Black cells
	fewer than 10 particles /cell	10 - 100 particles / cell	Innumerable particles

Table 1 . Semi-quantitative ranking method for histology

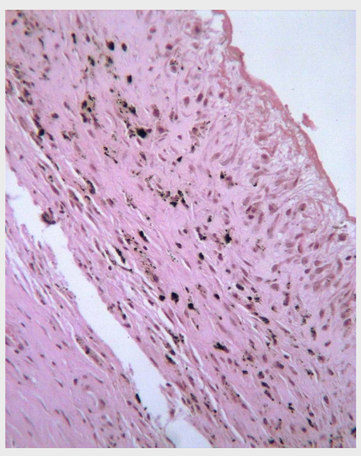

Fig 1. Tissue slide with a letter / number reference grid.
Fig 2. Area of tissue showing the variable amount of visible metal in macrophages, which complicates a single score

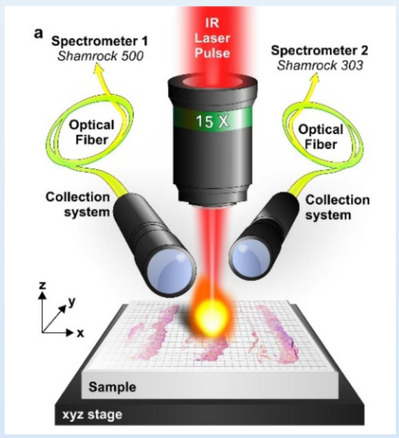

Fig 3. Schematic of LIBS equipment set up with paraffin block

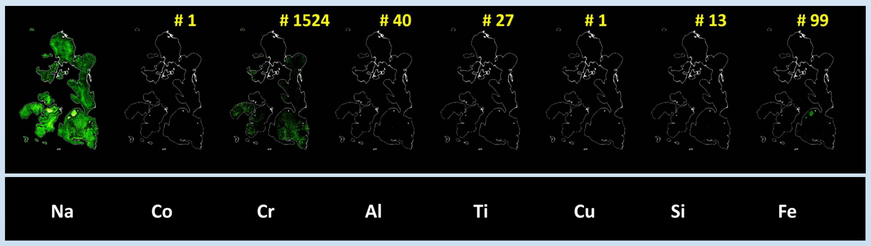

Fig 4. LIBS results output for multiple elements in 2511 (below)

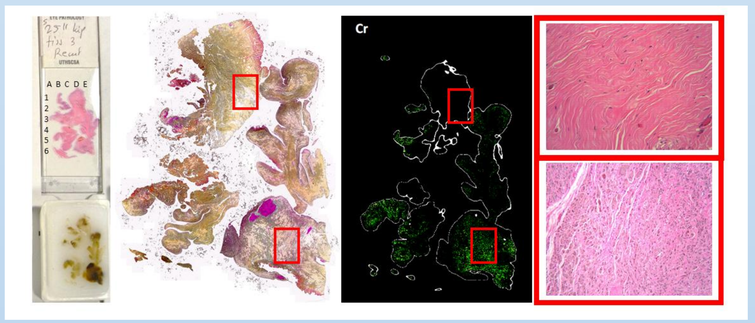

Fig 5. Corresponding tissue overview (left), LIBS map of Chromium counts (center) and histology of representative low Cr (fibrous area at top right) and higher Cr areas (dense brown macrophages at bottom right)

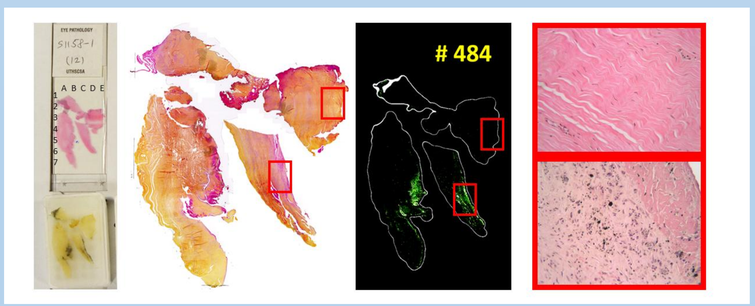

Fig 6. Corresponding tissue overview, LIBS map of Titanium counts and histology of representative low Ti (fibrous area at top) and higher Ti areas (dusty macrophages, bottom)

RESULTS

The LIBS findings and metal particle ranks are summarized in Table 2. By light microscopy, there was often a high degree of variability in the amount and appearance of debris within each slide (Figure 2). This made it difficult to choose an appropriate single rank score. No clear differences could be discerned between Ti and CoCr particles using light microscopy.
LIBS demonstrated the overall distribution of the various elements of interest as shown in Figures 4-6. The maximum level of each element was also provided (Table 2). Notably, in the tissues from CoCr components, Co was very low or absent while Cr was more abundant.

ID	Group	Co	Cr	Ti	Fe	Ranking
S2384	Corrosion	14	180	644	1454	0-3+
S2645	Corrosion	10	1166	1454	518	2+
S2511	High CoCr	1	1524	27	99	n/a
S2683	High CoCr	0	1310	5	7	1-2+
S1158	High Ti	0	4	484	149	0-3+
S1332	Low CoCr	0	2	16	16	0
S2307	Low CoCr	1	14	21	610	0
s2891	Low CoCr	0	29	12	774	0
ssu48	Primary	1	0	0	8	n/a
S2900	Ti & CoCr	0	1326	346	0	2+

Table 2. LIBS elemental counts and histology metal rankings

DISCUSSION

Arthoplasty-derived particles may include different metals, as well as taper corrosion products. The characterization of metallic debris in routine histological preparations is limited to the large or aggregated particles visible at the light microscope level. Ranking debris is complicated by their uneven distribution and does not provide elemental identification beyond the best estimates of the operator. LIBS technology overcomes these limitations. It allows for a more direct and quantitative elemental identification and can be used with direct reference to the H&E slides. In the series of cases described here, areas that appeared devoid of particles on the H&E slides were often demonstrated to contain elements of interest. LIBS can detect submicron, nanometer and ion-protein complex forms of biomaterials as well as endogenous particles that are undetectable at the light microscope level. LIBS has been previously applied to the identification of foreign material in subcutaneous nodular lesions and pigmented lymph nodes (3). It is a promising method for orthopaedic applications as well.

RELEVANCE

Accurately identifying and quantifying wear or corrosion products in tissues is not possible based on light microscopy alone. LIBS provides a visual multi-element image of reasonably large-sized tissue specimens (~ 6 cm^2), comparable with H&E images. LIBS data may prove to be more suitable for multivariate analysis than the currently used semi-quantitative rankings.

References

1.Sancey et al. Laser spectrometry for multi-elemental imaging of biological tissues. Sci Reports, Aug 2014. 2.Doorn et al. Tissue reaction to metal on metal total hip prostheses. CORR S329, 1996. 3. Busser et al. Characterisation of foreign materials in paraffin-embedded pathological specimens using in-situ multi-element al imaging with laser spectroscopy. Mod Pathol 2017