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Plasma cleaning of dental instruments

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Summary The theoretical risk of prion transmission via surgical instruments is of current public and professional concern. These concerns are further heightened by reports of the strong surface affinity of the prion protein, and that the removal of organic material by conventional sterilization is often inadequate. Recent reports of contamination on sterilized endodontic files are of particular relevance given the close contact that these instruments may make with peripheral nerve tissue. In this paper, we report the effective use of a commercial gas plasma etcher in the cleaning of endodontic files. A representative sample of cleaned, sterilized, files was screened, using scanning electron microscopy and energy-dispersive X-ray analysis, to determine the level of contamination before plasma cleaning. The files were then exposed for a short-term to a low-pressure oxygen–argon plasma, before being re-examined. In all cases, the amount of organic material (in particular that which may have comprised protein) was reduced to a level below the detection limit of the instrument. This work suggests that plasma cleaning offers a safe and effective method for decontamination of dental instruments, thus reducing the risk of iatrogenic transmission of disease during dental procedures. Furthermore, whilst this study focuses on dental files, the findings indicate that the method may be readily extended to the decontamination of general surgical instruments.

Introduction

The transmissible spongiform encephalopathies (TSEs), or prion diseases, are a rare group of fatal diseases, which in humans include, familial, sporadic and acquired Creutzfeldt-Jakob disease (CJD). The disease is characterized by accumulation of an abnormal form of prion protein in the central nervous system.[1] The risks of iatrogenic transmission of CJD is a topic of growing concern in healthcare as the infectious agent shows a marked resistance to conventional chemical and thermal decontamination procedures.[2] There is definite

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evidence of CJD transmission by growth hormone therapy and a number of surgical routes including dura mater graft and corneal implant procedures.[3,4] Cases arising from surgery, where contaminated instruments have been implicated, are much rarer. However, this should be considered in the context that the time lag between infection and appearance of the disease may be many years. The importance attached to the potential threat posed by transmission of CJD by surgical instruments has led the Department of Health in the UK to stress the importance of decontamination procedures.[5]

In contrast to the risks inherent in invasive neurosurgery the situation with regard to CJD transmission by dental instruments remains unproven. Epidemiological studies have so far indicated no correlation with dental procedures,[6] although they have been suggested as the causal link in two small CJD disease clusters.[7,8] While abnormal prion protein (PRP^{SC}) could not be detected in the dental pulp tissue of sporadic CJD patients,[9] no studies have been reported with patients incubating variant CJD where typically the levels of PRP^{SC}, and thus of infectivity, in peripheral tissues are higher. Indeed, Ingrosso *et al.*[10] have shown that the gingival tissue and dental pulp of hamsters infected with experimental 263 K scrapie prion by intradental injection are highly infective. Thus, there is a theoretical risk of CJD transmission in humans occasioned by reuse of dental instruments, such as endodontic files, which could come into intimate contact with peripheral nerves.[11]

Currently there is no accepted procedure for the removal of prion infectivity from surgical or dental instruments. Conventional processes such as autoclaving, exposure to ionizing radiation, formaldehyde treatment and sonication are ineffective. Smith *et al.*[11] have recently assessed the levels of gross contaminating matter remaining on endodontic files, after routine dental practice and dental hospital decontamination procedures, using a combination of visible and scanning electron microscopy (SEM). Their demonstration that significant amounts of material remain even after rigorous cleaning highlights the need to develop new methods of decontamination. Here we describe a preliminary study on the decontamination of endodontic files using radiofrequency (RF)-generated gas plasmas.

Although plasma cleaning has been little investigated in the medical context, it is a well-established technique for the elimination of organic material from surfaces. At one extreme, the method is used by the manufacturing industry for the solvent-free removal of machine oils from thermally robust machined components. At the other extreme, it is used by the semiconductor industry, to remove organic polymers from delicate electronic components.[12]

In contrast to conventional methods of cleaning, such as the use of solvents or aggressive chemicals, plasma cleaning leaves no residue, and when optimized, typically generates only CO_2, H_2O and N_2 as a gaseous waste. Gas plasma treatment has the potential advantages of having no toxic residue effects, reduced turnover time, and applicability for sterilization of heat- and moisture-sensitive instruments.[13-15] Its effectiveness has led to its use as a decontamination method from chemical and biological warfare agents[16,17] or from spores.[18] More significant in the present context is its reported effectiveness on routine surgical instruments.[19]

Material and methods

In this study, 15 hand-held endodontic K-files were obtained from a general dental practice. The dental files had each been used on at least one patient, and although some had been used on more than one patient, the specific histories of each file had not been recorded. All had been subjected to typical decontamination and sterilization methods.

Before plasma treatment, the files were subjected to visual examination. The initial detailed inspection of the files was conducted using SEM operating at 20 kV to provide a resolution of better than 5 nm (Philips XL30CP). The instrument incorporates a backscatter detector, which allows the imaging of specimens that have a mean atomic number difference of >0.1.

Secondary electron imaging showed that the condition of the files was broadly in line with that which has been recently reported.[11] Of the 15 samples, a significant proportion (5/15) had deposits that had a measured depth of over 50 μm under SEM analysis (Figure 1). The remaining files, whilst lacking gross amounts of contaminant material, were all found to contain much smaller quantities of organic matter that was typically around 1 μm deep, and several microns in diameter. In the latter cases, the metal surface of the files provides a high-contrast background for the detection of organic matter when imaged using backscattered electrons (Figure 2). The analysis of backscattered electrons allows much more sensitive and thorough detection, and subsequent analysis, of microscopic amounts of contamination than is possible with secondary electron imaging. Furthermore, it also shows that the

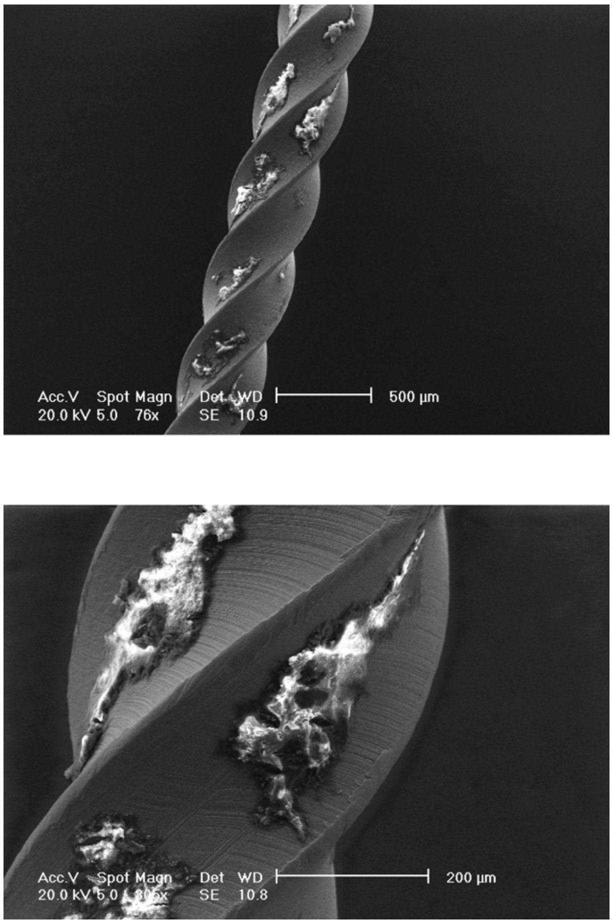


Figure 1 Scanning electron microscope images of an orthodontic file before plasma exposure. The foreign material was identified as nitrogenous organic material (probably protein), interspersed with small amounts of calciferous particles (probably dentine).

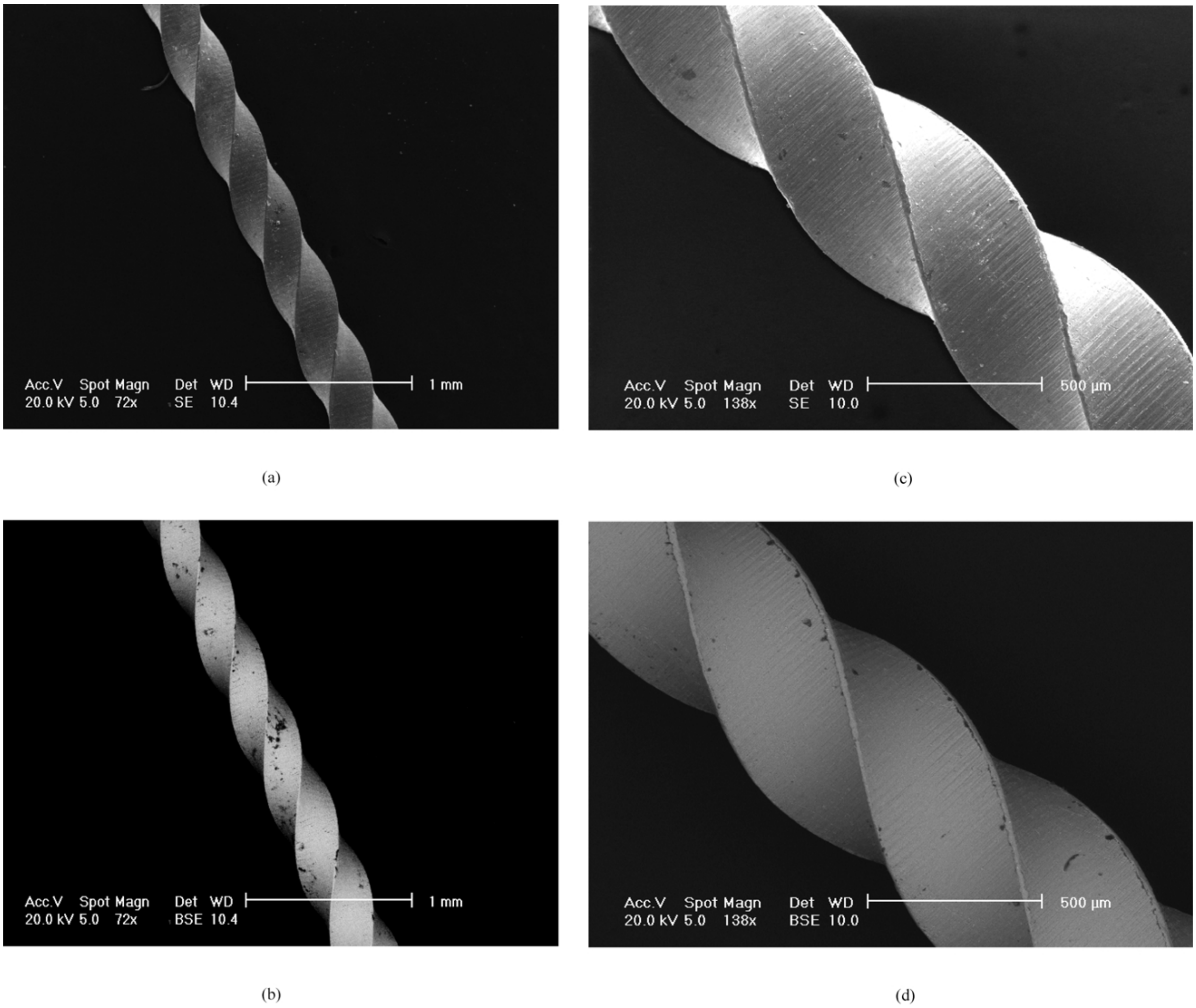
contamination coverage of the files is more significant than normal imaging methods would indicate.

In this work, biological or biochemical detection of specific proteins was not attempted. However, as amide links are a necessary component of any protein, the detection of carbon and nitrogen was instead taken to be an indicator of protein contamination. Sulphur is also a component of a number of common amino acids and its presence was used as a secondary indicator of proteins. To this end, the SEM was fitted with an X-ray analyser (Oxford Instruments Isis 300), capable of detecting elements of atomic number 6 and above, which was used for elemental analysis and imaging. The system is capable of detecting elements down to a limit of 1 ppm over a beam spot size of 5 μm diameter. In all the samples tested, the contaminant residues could be identified as either organic and potentially proteinaceous (as indicated by the presence of carbon, nitrogen, oxygen, and occasionally sulphur), or dentine fragments (as indicated by intense calcium and phosphorus signals).

Once analysed for the presence of organic matter, the files were treated with a low pressure oxygen plasma. The plasma unit used (Plasma Etch PE-200) is a versatile commercial instrument that is designed for, inter alia, the plasma removal of photoresistive material. Energy to sustain the plasma is supplied from a RF generator via a multi-electrode array upon which the files are placed. The plasma gas may be selected to obtain maximum etching of organic material whilst minimizing damage to the integrity of the substrate surface. The sample gas and power levels for this work were initially optimized by a number of trials using clean 314 stainless steel disks. These were coated with a fluorescein-based dye (synthesized in-house) and subjected to the plasma cleaning process. Comparison of the fluorescence intensity from the sample pre- and post-plasma treatment allowed the cleaning efficiency of given gas conditions and RF power to be assessed. In all cases, the temperature of the electrodes (and hence the samples) was held at a nominal temperature of 30 °C. For the work described here, a mixture of oxygen and argon flowed around the files at a pressure of <1 Torr, with RF excitation at a power level of less than 200 W.

Results

After being subjected to plasma cleaning, all the orthodontic files were visibly more lustrous and metallic. Under the SEM, the most heavily contaminated files showed no indication of the foreign organic material that was obvious using secondary electron imaging (Figure 3). Imaging of the surface using backscattered electron mode showed the almost total absence of the large dark patches that were clearly evident before the cleaning process. In all cases, careful elemental analysis of any remaining patches, which were invariably <10 μm in length, showed that they were either due to artefacts of the microscope (for example, shadowing of the electron beam), or were due to inorganic calcium and phosphate (probably due to remaining dentine). Carbon and oxygen were still present at all sample points over the whole surface, including those areas that were free from contamination, both before and after cleaning. The carbon and oxygen are assumed to be present in the form of interstitial atoms in the steel and as surface oxides, respectively. The absence of a nitrogen signal indicated that nitrogen-containing compounds (including proteins) were removed at least down to the detection level of the instrument.



(a) (c)

(b) (d)

Figure 2 Scanning electron micrographs of a lightly contaminated orthodontic file before plasma exposure. Foreign material, which is only poorly visible in secondary electron mode [(a),(c)], is clearly highlighted as dark patches in backscattered electron mode [(b),(d)].

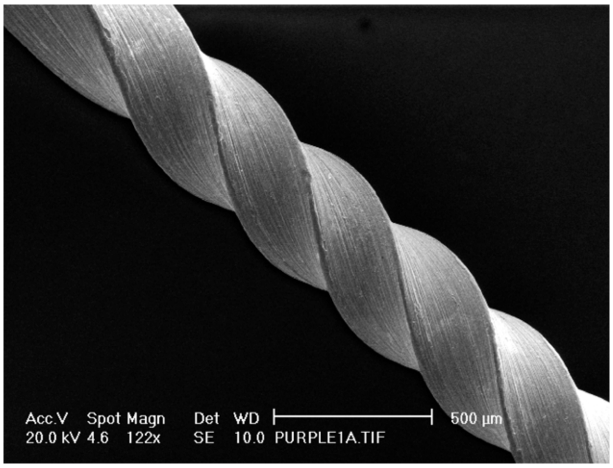


Figure 3 Scanning electron microscope image of the orthodontic file shown in Figure 1, following exposure to an oxygen–argon plasma.

In addition to analysing the reduction in organic/protein contamination, a 'visual' inspection was carried out, using the SEM, to determine whether there had been any significant changes to the file surface. In all cases, there was no evidence of substantial damage to the metal surface. Although quantitative mechanical tests have not yet been carried out, qualitative tests indicate that the flexibility and mechanical properties of the files are not noticeably changed through the use of this cleaning process.

Discussion

Despite the widespread use of plasma cleaning and plasma etching in industrial processes, including, ironically, the production of surgical instruments, its use in decontamination of surgical instruments has been limited. This preliminary study, which we aim to extend, has indicated that the use of gas plasma cleaning may be extremely beneficial in reducing the absolute amount of proteinaceous materials that may be transferred between patients when endodontic files are re-used. There is no reason to suppose that this method cannot be readily extended to other surgical instruments.

As there are no published data on the possible risks of prion disease transmission via such files, the presence of any protein material on these instruments represents a theoretical source of iatrogenic CJD transmission. This is of particular importance in view of the large number of patients whose treatments involve the use of reusable endodontic files. In the UK alone, over one million endontic operations are performed each year and 80% of dentists re-process endodontic files.[20]

The Department of Health advises that, despite the limited risk of iatrogenic infection, clinical sterilization and cleaning should be of the highest possible standard. The precise risks, if any, arising from this form of transmission are not known. It is known that the prion protein responsible for CJD is extremely robust. Any method, therefore, that dramatically reduces the amount of organic or proteinaceous matter on instruments that are widely reused,[21] over and above that obtained by conventional cleaning processes, is therefore potentially very useful, and worthy of further investigation.

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