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Ultrasound morphology of carotid plaque and its link with lipid:protein content and 3D microstructure of the plaque

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1. BACKGROUND

- **Ultrasound imaging of carotid plaques has revealed a spectrum of lesions ranging from plaques with predominantly echolucent (lipid-laden) properties to those which are densely echogenic (fibrous).**
- **Although fibrous plaques are essentially stable lesions, whereas lipid-laden plaques are prone to intimal tearing, it is still not possible to show any definite link between a specific plaque type and cerebrovascular events..**

2. OBJECTIVES

The main objectives of this study were to:

- **Investigate the correlation between plaque morphology and lipid: protein ratio in carotid plaque.**
- **Study the 3D micro-structure of the plaque, using confocal microscopy.**
- **Construct a blood flow model with a view to investigate how forces (resulting from fluid flow) interact with structural stability of carotid atherosclerotic plaque.**

3. METHODS

- **Carotid plaques were examined using bright-field and Laser-Scanning Confocal-Microscopy (LSCM) to generate 3D images.**
- **Lipid content was determined by drying the plaque first; the pre-dried sample was extracted directly in the Soxtherm. The protein of the sample was digested with boiling HCL to break the lipo-protein bonds. The digestion solution was filtered and the fat remaining in the filter after the drying period was extracted with petroleum ether. After the evaporation of the solvent, the residue was dried and weighed. The fat content was calculated from the difference between the initial sample weight and the weight at the end of the analysis.**
- **For protein content, the organically bound nitrogen content of the sample was digested with concentrated H2SO4, potassium sulphate, and a catalyst. The nitrogen thus is broken down to ammonium sulphate. By adding caustic soda in excess, ammonia is released by water steam distillation and trapped in a solution of boric acid. This solution is titrated against an acid solution.**

4. RESULTS

- **3D imaging of carotid plaques, using LSCM showed that echolucent plaques were predominately composed of lipid material, comprising necrotic core of amorphous debris and cholesterol clefts, with varying degrees of fibrous tissue present in all plaques (Figures-1 & 2). Regions of actual fibrous cap disruption and some ulceration were also seen in both echogenic and echolucent plaques; this is in addition to fraying of the fibrous cap with fibrous cap erosion and exposure of underlying necrotic core to lumen. Carotid plaque vulnerability (reduced fibrous cap thickness and large lipid-necrotic core with evidence of cracking) was also seen.**
- **Echolucent plaques have (lipid: protein ratio >1), whereas echogenic plaques have (lipid: protein ratio <1). Mixed plaques have wide range of lipid: protein ratio, depending on the predominant content (lipid Vs. protein), but generally it is slightly less than one.**
- **A blood flow simulation model (Figure-3) shows how blood velocity changes could occur in association with reduction in lumen diameter caused by the plaque.**

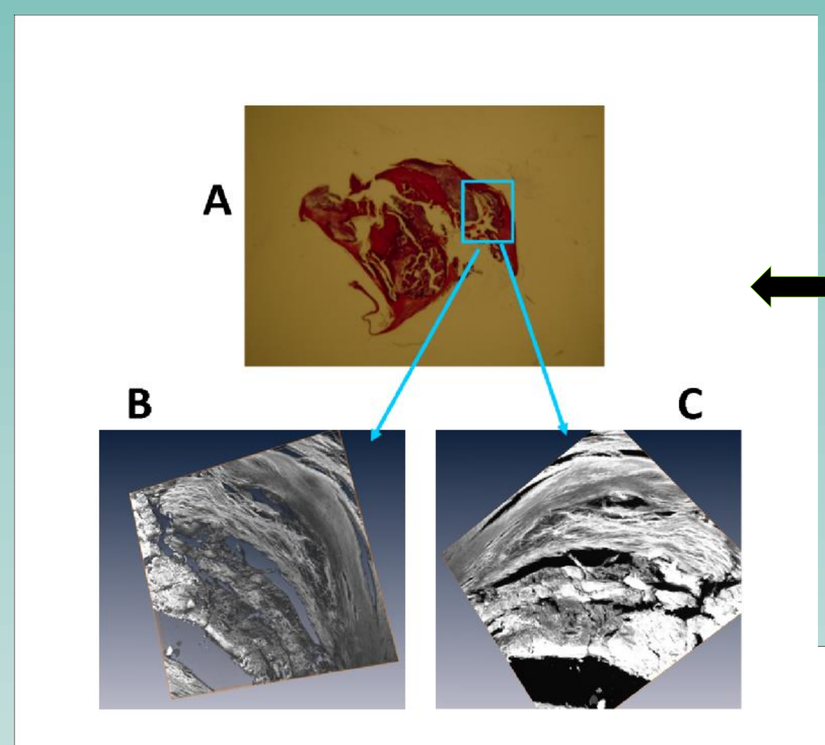
5. CONCLUSIONS

The clinical application of these findings lies in exploring using a hand-held Infra-Red device which can calculate Lipid: protein ratio of the carotid plaque –*in vitro*- immediately after surgery (in the operating theatre) to identify patients at higher risk of developing future plaques with a view to prevent or reduce the risk of stroke.

VAPODEST distillation system

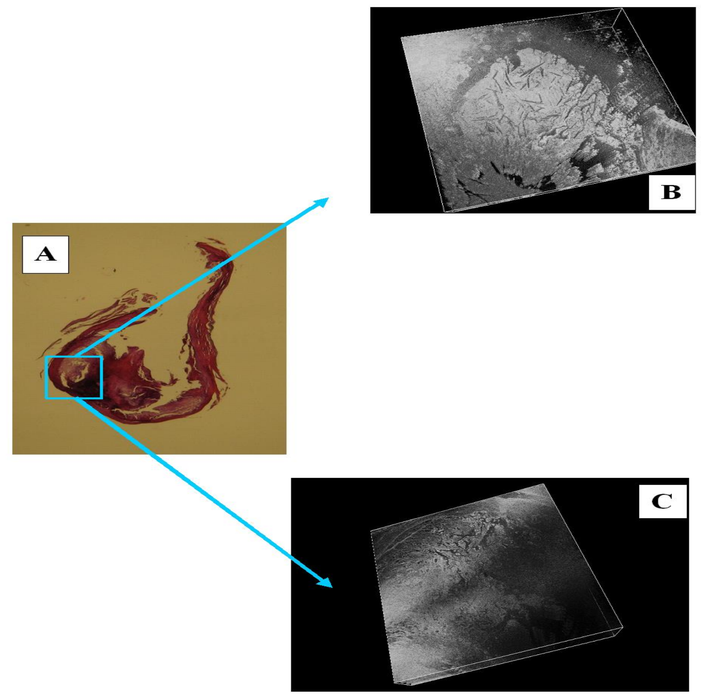
KJELDATHERM block digestion unit

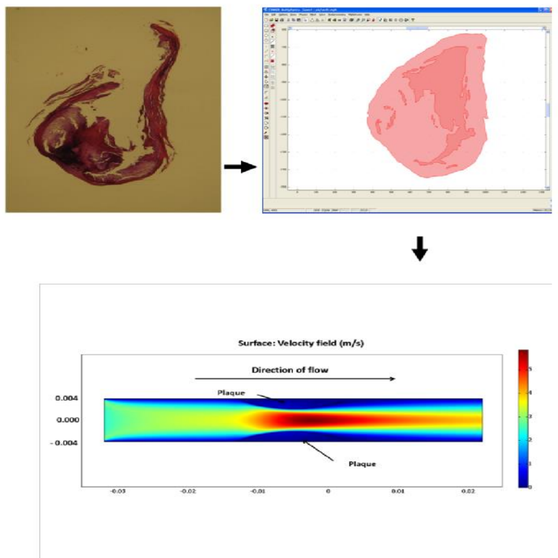
SOXTHERM – rapid extraction system for solid-liquid extractions



(Figure-1) Image (A) represents a low magnification image of cross section of a second carotid plaque specimen stained with H&E stain. Images (B & C) represent x20 objective orthoslice and intensity projection visualisation of part of carotid plaque two, using LSCM.

(Figure-2) Image of H&E stained plaque (A), showing amorphous pink material with slit-like "cholesterol clefts" of lipid material (B, C). There is overlying haemorrhage on the plaque, with a thrombus formed on top of the plaque (A). 3D visualization using IRIS Explorer.





(Figure-3) A low magnification image of H&E stained section of the plaque was enhanced using Paintshop Pro v9 (Jasc, USA) and converted to binary using MATLAB (The MathWorks Inc., USA). The image was then imported into COMSOL (Comsol AB) in order to simulate the effects of plaque in the artery. This enabled us to create a simple 2D flow models, using COMSOL v3.2.

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