Characterization of human melanomas by EPR Imaging

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Introduction
Malignant melanoma is a skin tumor characterized by the uncontrolled proliferation of melanocytes. The incidence of melanoma is rising each year. Nowadays, the cumulative lifetime risk for an invasive melanoma is estimated at 1/59 in U.S. For this reason, it is essential to develop new effective methods able to early detect melanoma. We demonstrated previously that radicals inside melanin pigments can be imaged used an Electron Paramagnetic Resonance (EPR)-based method (1) and that EPR spectrometry is an accurate tool to assess the growth stage of a pigmented tumor (2). As a proof-of-concept, the purpose of the present study was to assess the ability of EPR imaging to detect, localize and characterize melanin pigments inside samples mimicking melanoma with different invasiveness into the skin (samples differing by their Breslow index (thickness of the lesion) and Clark index (penetration of the tumor)). Moreover, we performed EPR Imaging on excised human melanoma samples.

Materials and methods
10 synthetic phantoms, simulating the shape of in-situ skin melanomas, were created with melanin extracted from sepia officinalis. These melanoma-like samples were measured by EPR spectrometry and imaging with a Bruker Elexsys system working in X-Band mode (9GHz). Typical EPR parameters were: microwave power: 2.6 mw; modulation amplitude: 2.5 G; sweep time: 30 seconds; number of scans: 10. 12 paraffin-embedded human skin melanoma samples (500 µm slice thickness; from the melanoma databank of Cliniques Universitaires Saint Luc) were measured and imaged using the same EPR system and parameters. 6 slices came from early growth stage melanoma (T1-T2) and 6 slices came from advanced melanoma (T3-T4).

Results
EPR imaging was able to detect and localize melanin pigments, both in synthetic phantoms and human melanoma slices. The images obtained from synthetic samples were faithful to their respective models as shown in figures 1.a and 1.b. The dimensions of these samples on the EPR images were very close to the real dimensions, with differences always below to 10% of the sample size. The quality of the images obtained from human melanoma slices was dependent on melanoma growth stage at the moment of excision. Overall, advanced tumors (T3, T4) with high pigmentation provided relevant images, while early detected tumors (T1, T2) were generally too small to provide a sufficient signal for EPR imaging. Figures 2.a and 2.b illustrate the comparison between a histological 5 µm slice and the EPR imaging obtained from a T3 human sample. The tumor area (enclosed in red) was well delimitated on the EPR image.

Discussion and Conclusion
In this work, we used for the first time EPR imaging as a tool to detect and localize melanin pigments inside human melanoma. The results are very encouraging as we showed that EPR imaging might be able to provide an accurate image of samples and thus allow the determination of the Breslow index (thickness of the lesion) and Clark index (penetration of the tumor), two crucial characteristics for the diagnosis of melanoma. Even if the sensitivity of the method has still to be improved to suit for early tumor stages, EPR imaging seems very promising to detect and characterize pigmented tumors.

References