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Microwave Assisted Decalcification with Recirculation of Temperature Controlled Solutions.

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Recent advances in microwave processing of tissue for histological analysis have shown its efficacy[1]. Improvements have been demonstrated in histologic detail, ease of processing and sample throughput time for both soft and hard (undecalcified) tissue [1,2]. Soft tissue has the advantage of being easy to sample and limited in size. This results in protocols of short duration with minimal sample heating at intermediate power settings (450W) and almost no heating(1 to 10EC) depending on solution type and volume. However, calcified tissue presents unique challenges to microwave assisted processing. Calcified samples are often large and cannot be trimmed without detrimental fractures through internal structures of interest (for example: the Organ of Corti of the cochlea embedded within the temporal bone). These large samples also present a significant diffusion gradient against the removal of hydroxyapatite. In non-microwave assisted protocols, samples require daily changes of large volumes of solution over several weeks to months to achieve decalcification at room temperature.

Several studies have been published for microwave assisted processing of calcified tissue[3,4,5]. Variables include decalcifying solution type and concentration, time, temperature and type of microwave processing. Microwave factors have varied with type of oven (household to laboratory) wattage settings, temperature control and magnetron cycle times. These studies have led to a disagreement over the effect of solution temperature during microwave irradiation. The goal of this study was to evaluate the effect of microwaves on the rate of decalcification independent of temperature.

We compared the decalcification rate for standardized samples of gerbil calvarium by the following methods: 1) Constant rotation of 10 ml vials (16 rpm at 30E of inclination) at 20 EC with daily changes of 5 ml of decalcifying solution (standard non-microwave processing). 2) Constant recirculation of 9 L of decalcification solution at 20 EC. 3) Constant recirculation of 9 L of decalcification solution at 20 EC with simultaneous exposure to microwave irradiation using a Pelco Model 3470 laboratory microwave oven. Random samples from each group were removed at various intervals, dehydrated and embedded in epon/araldite plastic. Sections were cut at 1 um and evaluated for the degree of decalcification. Microwave exposure significantly increased the rate of decalcification, independent from temperature, when compared with the other two methods.

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Fig. 1 Fig. 2

Fig. 1. Cross section of gerbil calvarium after 21.5 hours of constant recirculation of decalcifying solution at 20 EC. Note remaining unstained undecalcified bone

Fig. 2. Cross section of gerbil calvarium after 20 hours of constant recirculation of decalcifying solution at 20 EC and constant microwave irradiation. Note complete decalcification.

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