

The Evaluation of Histological Methods for Biodegradable Magnesium

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INTRODUCTION: The histological evaluation is an essential tool used in the field of orthopedics to examine the biocompatibility, bone fracture healing, and articular cartilage repair of implants. The examination of bone biopsies near the implanted sample provides valuable information on bone structure, remodelling, and turnover. Recently, there have been significant advances in development of biodegradable magnesium and its alloys as orthopedic implant materials. However, there are currently no standardized methods available in the literature to reflect on to perform the histological evaluation of the magnesium alloys and the unique corrosive characteristic of magnesium often hinders the accurate observation.

The purpose of this study was to evaluate the four most commonly used bone histological staining methods (Goldner's trichrome, Toluidine blue, von Kossa with Gieson counterstain, Villanueva stain) to observe their effectiveness when used on magnesium.

METHODS: Four most commonly used staining methods for bone histologic analysis were performed using 0.25 mm thick and 1 mm long 99.9% pure magnesium samples to measure the weight loss after each staining procedure. Same standard protocol procedures were used for the slides made from in vivo test using New Zealand White Rabbits.

RESULTS: Goldner's trichrome stain showed most corrosion with $35.86 \pm 4.15\%$ weight loss. Toluidine blue, Villanueva and von Kossa stained showed $-1.32 \pm 0.88\%$, $-0.11 \pm 0.81\%$, $1.79 \pm 1.60\%$ weight loss, respectively. Negative weight loss of Toluidine blue and Villanueva stain is due to the stain residue left on top of the specimen. From the procedure of Goldner's trichrome method, Weigert's hematoxylin solution (mixture of hematoxylin A and B) caused most severe corrosion. After 10 minute of immersion, pure magnesium specimens lost $33.04 \pm 2.14\%$ weight in hematoxylin A and $37.55 \pm 3.02\%$ weight in hematoxylin B.

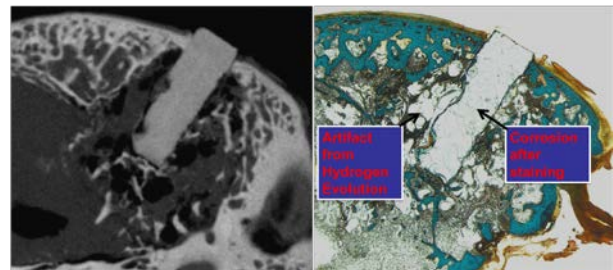


Fig. 1: Results of Goldner's trichrome stain

Histologic analysis of Mg alloys from in vivo test using Goldner's trichrome staining demonstrated corrosion of degrading implant sample after the stain was applied. Toluidine blue staining and Villanueva staining did not show the corrosion of specimen.

DISCUSSION & CONCLUSIONS: Goldner's trichrome staining method is the most commonly used staining procedure for the implant/bone evaluation. It provides accurate and precise analysis of new bone formation around the implanted samples made out of inert metals such as titanium and cobalt chrome alloy. However, severe corrosion of intact Mg alloy specimen was observed for Goldner's trichrome method and hydrogen gas generated from the corrosion hindered the accurate evaluation. This is due to the Ferric Chloride and Hydrochloric Acid in Goldner's trichrome (Weigert's hematoxylin solution), which are both extremely corrosive.

The result from this study suggests that the Toluidine blue and Villanueva staining methods, which contain basic solutions, are the ideal staining procedure to accurately evaluate the in vivo application of magnesium and its alloy.

REFERENCES: ¹ Y. An (2003) *Handbook of Histology Methods for Bone and Cartilage*, Humana Press, Inc

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