Comparative staining technique for calcified bone with metallic implants

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Unveiling the growth pattern of bone tissue after implantation, may help the development of implants with specific features, besides establishing recovery time for each step during bone repair. This study aimed to identify the bone remodeling in rats during repair after surgical implantation of porous metallic implants with histological approach over calcified bone-implant samples using different staining methods.

Implants of Ti-13Nb-13Zr alloy with mean porosity of 30% and cylinder shaped were surgically positioned in femurs of Wistar rats males of 10 weeks old. The animals were euthanized on day 28 and bone-implant samples were fixed with formalin solution, the blocks of calcified tissue samples were prepared using Technovit® 9100 NEU resin and the histological slides from the blocks were performed using Isomet cutter and finally trimmed with sandpaper to achieve the desirable thickness to perform staining and further analysis. The histological evaluation were made using bright field of unstained (U) slides and hematoxilin-eosin (HE), toluidine blue (TB) and Masson's trichrome (MT) stained slides. To perform all the staining and analysis of the slides the resin wasn't washed out as with paraffin preparations.

The morphological analysis was performed by optical microscopy, which followed from U, MT, HE, TB. Each of these preparations showed different features of bone repair. From all techniques the osteocyte nucleus was always distinct and recognizable even with U slides, due to the resin impregnation and thickness of the slides. In comparison to the three staining methods used the HE marked well the differences between the osteocytes nuclei in purple and the surrounding bone matrix in pink, for the MT stained slides the bone matrix got stained by two shades of blue, light blue for a new formed bone, and dark blue for old bone, which pointed out the grade of bone remodeling that took place after implantation. From TB slides the highlight were the metachromasia in bone matrix, which presented as pinkish color in contrast to the blue staining of other structures. Besides differentiating the several stages of bone maturation and remodeling was possible to evaluate the cell migration and differentiation towards the center of the cylinder shaped metallic implant as well as the intimacy of the bone-implant interface. The content of the bone-implant interface without fibrous capsule which could be evident by HE, MT and TB showed a successfully osteointegration.