Studying the Effect of Different Biomaterials on Healing Process in Bone Injury Model Using Microscopic MRI and Micro CT

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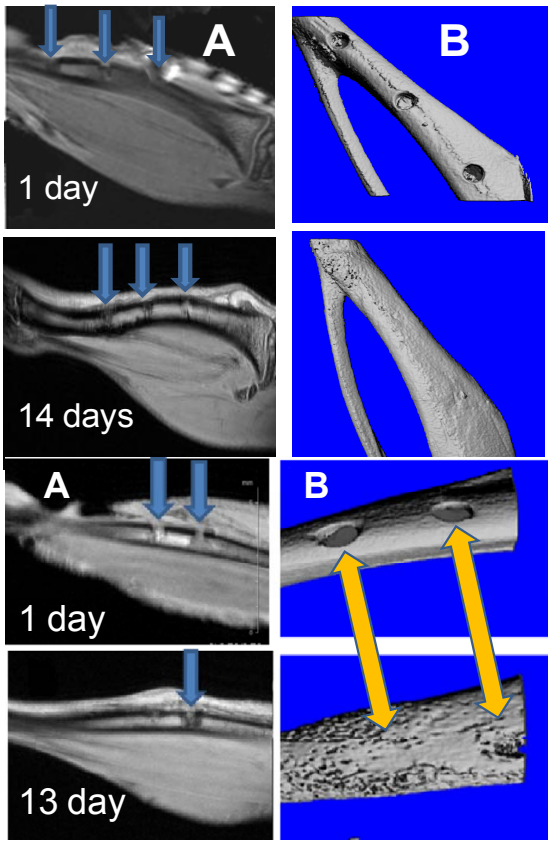
INTRODUCTION: Biomaterials such as hydroxyapatite (HA), matrigel and purecol are applied in tissue engineering based therapies. These biomaterials are used as carriers for stem cells (matrigel or purecol [1]) or contribute to bone formation (HA [2]). It was shown that transplantation of cultivated bone marrow stromal cells in collagen gel caused 30% increase in bone regeneration when compared to controls after 120 days [3]. Therefore, studying the effects of these biomaterials on bone healing is crucial. For our study, we used a burr hole fracture model in mice [4].

In order to assess bone healing, micro-imaging methods are needed. We combined µCT which was used previously for this purpose [5] with microscopic MRI which to our knowledge has never been used for this purpose, to assess the influence of different material on bone healing. In this study, MRI was used to image the whole leg while µCT has been processed to show bone only.

METHODS: Female mice were divided into 2 groups. Three holes were drilled in the medial aspect of the tibia. The different burr holes were filled with different compounds to compare the effects on healing. The proximal hole was left empty; the centre hole was filled with HA. In group 1 the distal hole was filled with matrigel while in group 2 it was filled with purecol. Mice were imaged on days 1 and 14 post surgery using MR and µCT. For MRI, RARE images were acquired (TR/TE=2000/7.5ms, NA=12, matrix=256x256, slice=0.5mm, pulse angle180º, RARE factor=4, resolution=100x50x500µm, FOV=2.56x1.28cm) on a 9.4T Bruker Avance system. µCT images were Gaussian filtered (sigma=1.2, support=1) and segmented so that only voxels with attenuation greater than 25.5% of the maximum gray scale value were included. µCT resolution= 12.5µm, Integration time=200ms, Voltage= 45KVp on a vivaCT (Scanco Medical).

In one mouse only two holes were drilled in the tibia. The proximal hole was filled with embryonic stem cells (ESCs) + purecol, and the distal hole was left empty. MRI and µCT scans were acquired on days 1 and 13. The µCT parameters were; resolution=10µm, FOV=2.12, integration time=200ms, measuring projection=2000.



RESULTS: In general, the HA, matrigel and purecol loaded holes showed healing that was equal to that of the empty hole (Fig 1). At 1 day post surgery, both the MRI and µCT showed multiple holes. By 14 days post surgery, there was heterogeneity in the visualization of the holes in that MRI often showed residual damage when the µCT sometimes showed an image that appeared to be healed bone (Figure 1, comparing 1 and 14 days). In one animal with both purecol and stem cells, at 14 days clear healing was observed by µCT and MRI in the unloaded hole while the treated hole showed limited healing (Fig 2).

Figure 1 MRI and µCT of experimental bone injuries loaded with different matrices (matrigel, HA and control or empty hole). **Column A.** MRI, **Column B**. µCT both at 1 and 14 days post surgery. By 14 days, the µCT in this subject showed healing in all holes while the MRI still showed damage within the bone.

DISCUSSION: A previous study shows that HA contributes to healing in bone [2]. Our study confirmed that on average, no implant materials inhibited healing. This is important as it paves the way for the use of either of the implant materials as a support matrix for stem cell therapy. The MRI clearly shows added information to the µCT in that heterogeneity of healing was detected with MRI when it was not apparent with µCT.

Figure 2. MRI and µCT of one animal with embryonic stem cells plus purecol in the right hole and no implant in the left hole. **Column A.** MRI, **Column B.** µCT both at 1 and 13 days post surgery. Both methods showed the control hole to be healed and the implant hole to be only partially healed.

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