Characterization of the Immune Response Associated with Bone Regeneration Induced by Fibrinogen Implants

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Statement of Purpose: Inflammatory response is a natural event following biomaterials implantation. The concept of "fighting inflammation" has been gradually shifting to "modulating inflammation"[1]. Our team has previously shown that the incorporation of a pro-inflammatory and pro-healing molecule, fibrinogen (Fg), in chitosan scaffolds is able to modulate the systemic immune response while improve bone regeneration[2]. In this study we addressed the potential of Fg 3D scaffolds to promote bone regeneration. Also, we investigated the local and systemic response to these scaffolds at early and late time-points after implantation.

Methods: Fg scaffolds were prepared by freeze-drying method as previously described by our group for other materials[2]. Animal work was approved by DGAV. Each Wistar rat (3 months) suffered a femoral critical size bone defect, which remained empty or received Fg scaffolds (n=12/group). After 6 days (early) and 8 weeks (late) post-implantation, animals were sacrificed and blood (BL), lymph nodes (LN), spleen (SP) and femurs were collected. Some of the femurs (n=5) were processed for histology (H&E and Masson's trichrome staining) and were observed in a light microscope Olympus CX31. Gene expression analysis of inflammatory and bone-related markers was performed at the defect site in the remaining femurs (n=7). Immune cell populations from BL, LN and SP were freshly isolated and stained against CD45R, TCR, CD4, CD8, CD161a, CD18, CD11b/c and MHC class II before data acquisition in a FACSCanto. The results were analyzed with FlowJo. Pro-inflammatory cytokines in plasma were quantified by ELISA. Statistical analysis was performed using Prism software.

Results:

Histological evaluation showed that 8 weeks after implantation Fg scaffolds promoted bone regeneration, being replaced by bone tissue. At early time points (6 days), there was an increased infiltration of connective tissue at the periphery of the defect in the Fg-group correlated with a decreased defect size.

Analysis of the local immune response revealed that at 6 days post-implantation, there was significant increase in IL-6 and IL-8 mRNA levels for both, empty- and Fg-groups of animals when compared with non operated animals, while IL-1β remained constant. An increase of VEGF mRNA in the empty group was also detected. At the same time-point post-implantation, TGF-β mRNA level at the bone defect as well as its concentration in plasma was increased in the "empty"-group of animals, while at 8 weeks post-implantation no significant differences were observed between the plasma of the different groups of animals. The pro-inflammatory cytokines TNF-a, IL-6 and IL-17a were not detected in plasma, at either time point.

At the systemic level, Fg scaffold implantation correlated with changes on the proportions of immune cells. A reduction of B cells, with a concomitant increase on T cells, was particularly significant in SP at 6 days post-implantation. Fg also led to less myeloid, NK and NKT cells in BL. Interestingly, Mac-1+ (CD18+/CD11b/c+) cells were significantly decreased in the BL, LN and SP of animals implanted with Fg scaffolds. Furthermore, a lower percentage of TCR^{dim} T cells and of MHC class II+ CD11b/c+ cells was found in the BL and SP of animals with Fg implants, indicating towards a lower activation of immune cells. After 8 weeks of implantation, there was again a reduction of B and myeloid cells and an increase in the percentage of T cells on Fg implanted animals.

Conclusions: Fg scaffolds promoted bone formation after 8 weeks of implantation. A mild inflammatory response was observed 6 days after injury, either with or without Fg implants, when compared with non-operated animals. Alterations in immune cell proportions and their activation status, at an early time-point indicate there is a reduced inflammatory response in the presence of Fg scaffolds, which correlate with increased bone formation at a later stage.

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References:

1. Mountziaris, PM. et al. Tissue Eng Part B Rev 2011; 17: 393-402-
2. Santos SG. et al. Acta Biomater 2013; 9: 7209-7217.