Biocompatible Ex Vivo-Induced Designer Fracture Hematoma for the Healing of Segmental Bone Defects

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Purpose: The fracture hematoma is a natural fibrin scaffold and is crucial for the initiation of bone healing, serving as a reservoir for growth factors and a space for (stem) cell infiltration. However, it is not clear if hematomas in normal fractures, which heal, differ structurally from the ones in large bone defects, which do not heal. Therefore, the aim of this study was to determine the structural properties of hematomas in normal and large bone defects, and to assess if the structure of a normal fracture hematoma can be mimicked ex vivo using a coagulating factor (CF) to enhance the repair of large bone defects.

Methods: Defects of 1 and 5 mm were created in the rat femurs. In vivo fracture hematomas and whole blood were collected on day 3. CF was added at various concentrations to citrated blood to form ex vivo hematomas. Scanning electron microscopy (SEM) was used to determine structural properties. Alamar Blue assay was used to assess the effect of the CF on the cell viability of rat bone marrow mesenchymal stem cells (MSCs).
Results: SEM images of in vivo hematomas revealed that fibrin fibers in 5-mm defects (320 ± 64 nm) were 50% thicker compared to 1-mm defects (209 ± 20 nm) 3 days after surgery (Fig. 1). Increasing concentrations of CF led to thinner fibrin fibers in ex vivo hematomas, which ranged from 173 ± 9 nm at the lowest concentration to 93 ± 3 nm at the highest. Cell proliferation rate decreased with an increasing concentration of CF, showing a 6.9 ± 0.9-fold growth at the lowest concentration, a 3.3 ± 0.3-fold at the highest, and a 7.6 ± 1.1-fold increase without the CF.

Conclusion: This study is the first to quantify in vivo structural differences of hematomas between normal fractures and large bone defects. Likewise, using CF we were able to modulate the structure of fibrin fibers in ex vivo-induced hematomas. The cell viability assays confirmed biocompatibility of the CF at lower concentrations. Taken together, this study showed that mimicking the structure of normal fracture hematomas could be the first step towards developing new treatment strategies that improve the healing of large segmental bone defects.