MECHANICALLY DRIVEN CELLULAR SELF-ORGANIZATION AS A MECHANISM DRIVING LARGE BONE DEFECT HEALING

Ehsan Soodmand (1,2), Aaron Herrera (1,2), Georg N. Duda (1,2), Ansgar Petersen (1,2), Sara Checa (1,2)

1. Julius-Wolff-Institute, Charité-Universitätsmedizin Berlin, Germany; 2. Berlin-Brandenburg School for Regenerative Therapies, Berlin, Germany

Introduction

Bone has a unique capability of healing without any scar formation. However, if the fracture gap exceeds a certain size (a large bone defect), its healing capacity is insufficient leading to a non-union. One peculiarity of this healing situation is that healing leads to limited bone formation in the bridging direction and results in closure of the medullary cavity. However, the mechanisms behind this bone tissue patterning remain poorly understood. It is known that mechanics drives cellular self-organization [2], however it remains unknown how the mechanical conditions within a large bone defect might affect cellular organization and as a result tissue patterning. The aim of this study is to investigate how the mechanical feedback between cells and the extracellular matrix (ECM) could influence early cellular organization within a large bone defect.

Methods

A combined *in silico/in vitro* approach was established to investigate the mechanical regulation of cellular self-organization in large bone defect healing [2, 3]. An *in vitro* microtissue clamp setup was established to study cellular organization under controlled mechanical conditions mimicking the geometrical constrains in a bone defect. An agent-based computer model with stiffness-driven cellular organization rules was combined with finite element (FE) analyses to determine the local mechanical signals surrounding the cells and their influence on cellular organization (Figure 1). Three different configurations were investigated: (A) Large gap (5mm, based on a rat model) with cells initially seeded at the gap's boundaries, (B) Large gap with cells initially seeded homogenously in the gap, and (C) Small gap (1mm) with cells initially seeded at the boundaries of the gap (Figure 1a-c). The cellular organization in the gap area was quantified 7 and 14 days post-seeding in two different regions of interest (ROI), the periphery region (close to the boundaries) and the middle of the gap area.

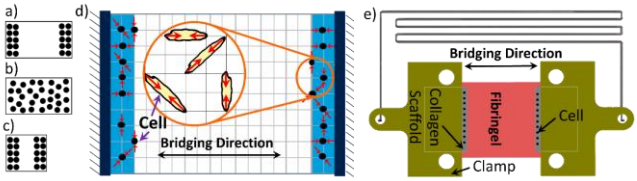

Figure 1 – a) Sketch of configuration "A" b) Configuration "B" c) Configuration "C". d) Schematic illustration of FE model representing configuration "A". e) Schematic representation of the in vitro system in configuration "A".

Results

In silico, comparison of cellular self-organization in the small and the large gap showed that stiffness-driven cellular migration results in altered cellular self-organization for different gap sizes. In the small gap, a fast cellular organization takes place in the bridging direction, whereas in the large gap, close to the gap ends, cells were organized dominantly perpendicularly to the bridging direction. In comparison, initial spatial cell position showed an influence on cellular self-organization. Both *in silico* and *in vitro*, a homogeneously seeded large gap led to a fast organization of the cells in the bridging direction in all ROIs. (Figure 2).

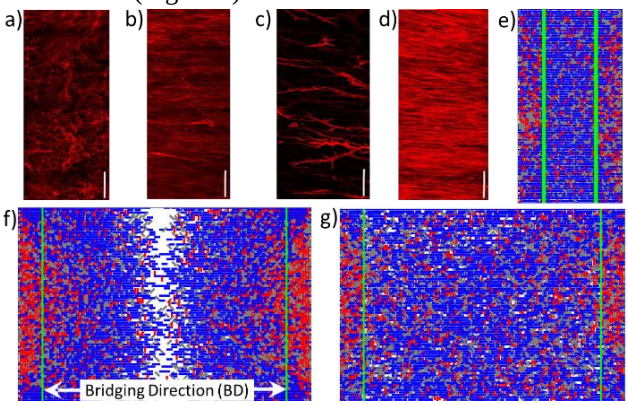

Figure 2 - Cellular organization in the in vitro experiments (a-d) and in silico (e-g) after 14 days. a) Periphery region in configuration "A". b) Periphery region in configuration "B". c) Middle of gap in configuration "A". d) Middle of gap in configuration "B" e) Configuration "C". f) Configuration "A". g) Configuration "B". The experimental cellular organization is based on f-actin signal, scale bars are 200µm. In in silico figures blue, red, and grey represent the cells oriented in the BD, perpendicular to BD and diagonal to BD, respectively.

Discussion

We showed that both gap size and the initial location of the cells influence cellular self-organization and that this organization can be explained by the mechanical communication between the cells through the extracellular matrix. Our results show that cells orient perpendicular to the bridging direction in a large gap, which is similar to the organization of early extracellular matrix in large bone defect healing, where the ECM encapsulates the marrow cavity [1]. Future studies will investigate the consequences of spatiotemporal cellular organization on ECM pattering with the final goal of using this knowledge to design strategies for the treatment of large bone defects.

References

1. Petersen, et al., Nat Commun, 9(1): p.4430.2018.
2. Checa,et al., Biomech. Model. Mechanobiol, 14(1):1-13; 2015
3. Könnig D, J Tissue Eng Regen M, 12(1):265-75; 2018

Acknowledgements

This work was financially supported by "Friede Springer Stiftung" and Charité - Universitaetsmedizin Berlin.