A computational tool to elucidate the mechanobiological regulation of limb development

Reporting

Project Information

COMPLIMB

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Periodic Reporting for period 2 - COMPLIMB (A computational tool to elucidate the mechanobiological regulation of limb development)

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Summary of the context and overall objectives of the project

Congenital limb malformations affect between 0.2-0.9% of live births in Europe. The correct formation of limbs and their joints is critical for healthy function in adult life. Yet, important aspects of joint formation are still not fully understood. For example, forces from muscle contractions and motion are critical to creating a properly shaped joint, but it is unclear how cells sense and respond to these mechanical cues.

Understanding the role of mechanical stimuli in joint formation can help identify when and how pathological conditions arise that result in malformations, and potentially provide clues on how to avoid or correct joint deformities. Ultimately, a better understanding of joint formation could inform therapies to correct joint deformities, as well as contribute to the development of preventive strategies for congenital limb defects.

The main goal of the CompLimb project was to determine the role of mechanical stimuli and mechanosensitive growth regulators driving joint formation in vertebrates. To achieve this goal, we used a combined experimental and computational approach. Limb formation has been widely studied in axolotl salamanders (Ambystoma mexicanum), as they regrow limbs throughout their life. Experiments on regenerating axolotl limbs provide information on how altering the cell's ability to sense and respond to mechanical stimuli affects joint shape as well as the location and timing of molecular expression critical to joint formation. Predictive computational models informed by the experimental findings allow us to explore potential physical mechanisms of normal and pathological joint formation.

Work performed from the beginning of the project to the end of the period covered by the report and main results achieved so far

We performed experiments in which we blocked the ability of cells to sense and respond to mechanical stimuli (mechanosensitivity) during the joint morphogenesis phase of forelimb regeneration in axolotls. Joint morphogenesis occurs after the joint has appeared (cavitation) and is the process by which the joint gets its final shape. After the forelimbs had regrown, we collected and imaged them using a microscopy technique that allows 3D imaging of the whole joint. We then counted the number of proliferating cells and quantified joint shape for the control and experimental groups. Our analysis, which focused on the humerus bone rudiment (the long bone of the upper arm), shows that animals with impaired mechanosensitivity had reduced cell proliferation and an altered elbow joint shape.

In parallel, we developed a finite element model of growth at tissue level to study how limb motion might regulate humerus morphology during joint morphogenesis. In this mathematical model of the limb, we assumed that pressure promoted growth. This pressure changes over a flexion-extension cycle of the elbow. We modelled the geometry and loading conditions of a generic axolotl humerus based on experimental data, calculated pressure during elbow flexion, and grew the model in proportion to the pressure. The computational predictions of the shape of the control and mechanosensitivity-impaired cases matched the experimental findings. Our results suggest that

pressure may be an important stimulus in regulating local tissue growth during joint formation. A detailed description of the project's finding was recently published in Proceedings of the Royal Society B, with doi:10.1098/rspb.2022.0621.

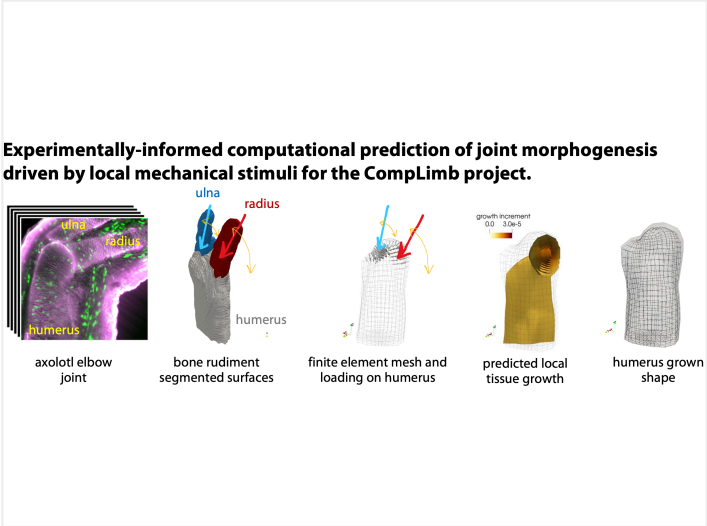
Progress beyond the state of the art and expected potential impact (including the socio-economic impact and the wider societal implications of the project so far)

To date, joint shape has been studied based on linear measurements of the humerus bone rudiment. Analysing shape using 2D measurements means important information of the 3D structure such as volume or growth distribution might be lost. 3D measurements have not been possible until recently, due to limitations in the existing imaging techniques. Based on our new 3D whole limb microscopy images, we have developed a methodology to map the humerus surface onto a standardized reference surface. We then normalize the mapping and perform statistical analyses on the computed areas and volumes that we extract from each limb's surface map. Our data analysis method is the first to allow for full 3D quantification of bone shape.

Our poroelastic computational model incorporates, for the first time, the effect of dynamic loading on local tissue growth. Previous models of joint morphogenesis used elastic materials, which cannot account for the fluid flow and, hence, the dynamic pressure maps that change over a loading cycle. These pressure maps are the ones dictating growth in our model. In addition, for the first time, we have developed a joint morphogenesis model informed by and validated with experimental data.

We also started to analyse the results of the latest experiments in which we used a new technique to image the expression of morphogenetic biomolecules in healthy regenerating axolotl forelimbs around the time that the joint appears (cavitation). Studies to date have been limited because techniques to quantify molecular expression loose spatial patterning, while methods that allow visualizing their expression in space have been, thus far, exclusively in 2D sections and non-quantifiable. Our new technique will allow mapping in 3D the location of these biomarkers. We will use the experimental results to develop in parallel a new computational model to predict joint cavitation. We have started to develop this model, which uses reaction-diffusion equations to predict the emergence of Turing patterns in 3D space.

Next steps beyond the end of the CompLimb project will include incorporating a growing domain as well as the potential mechanical stimuli influencing the expression of these morphogens. Combining experiments and modelling will provide a framework for exploration with the aim of better understanding the exact biochemical conditions required for healthy limb formation and, more specifically, how the biophysical environment influences these molecular components responsible for growth. The findings derived from the CompLimb project will contribute to a better understanding of vertebrate joint formation. This knowledge might ultimately have an impact on the development of therapies to treat joint deformities or approaches to prevent congenital malformations.



Experimentally-informed computational prediction of joint morphogenesis driven by mechanical stimuli

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