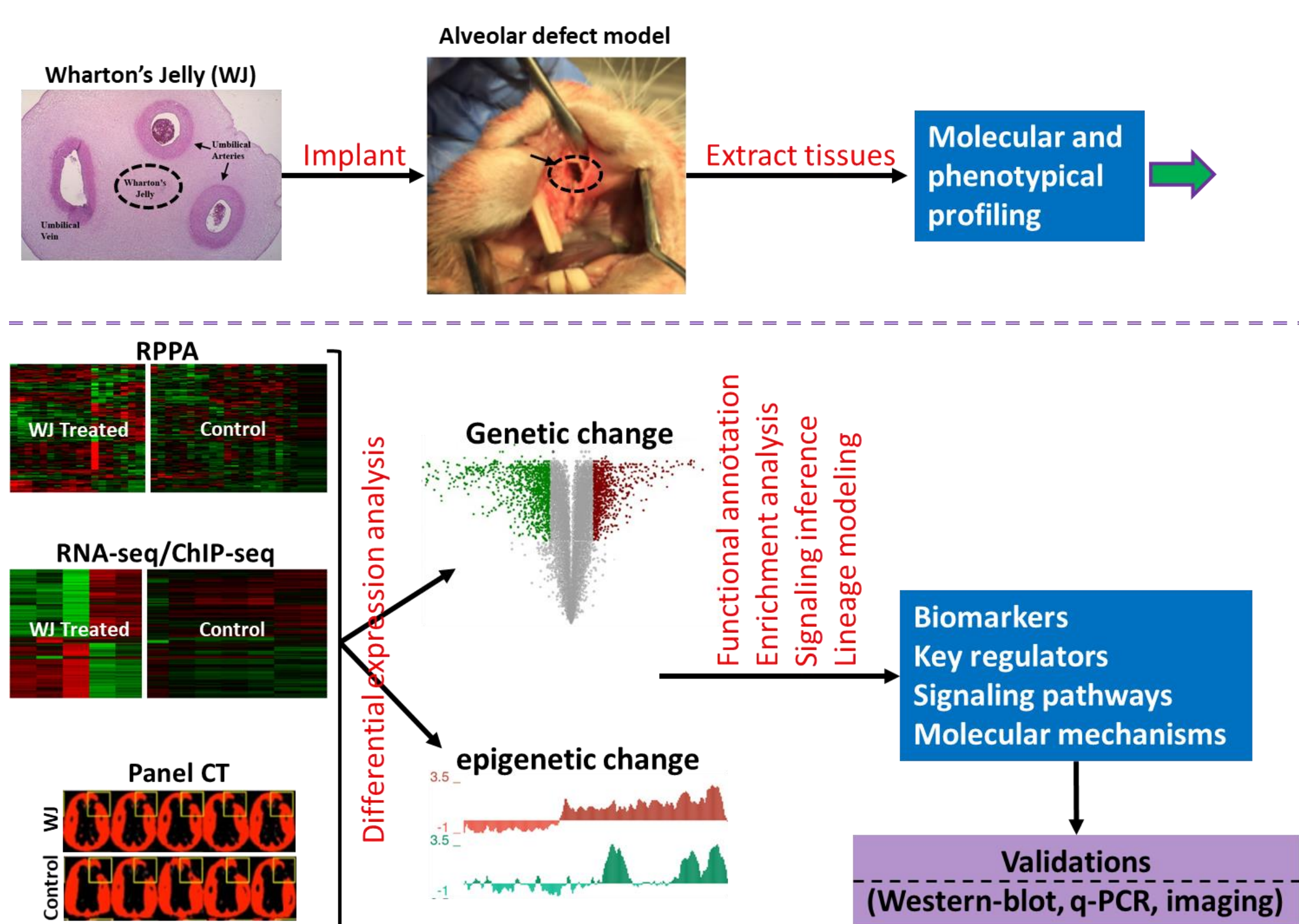
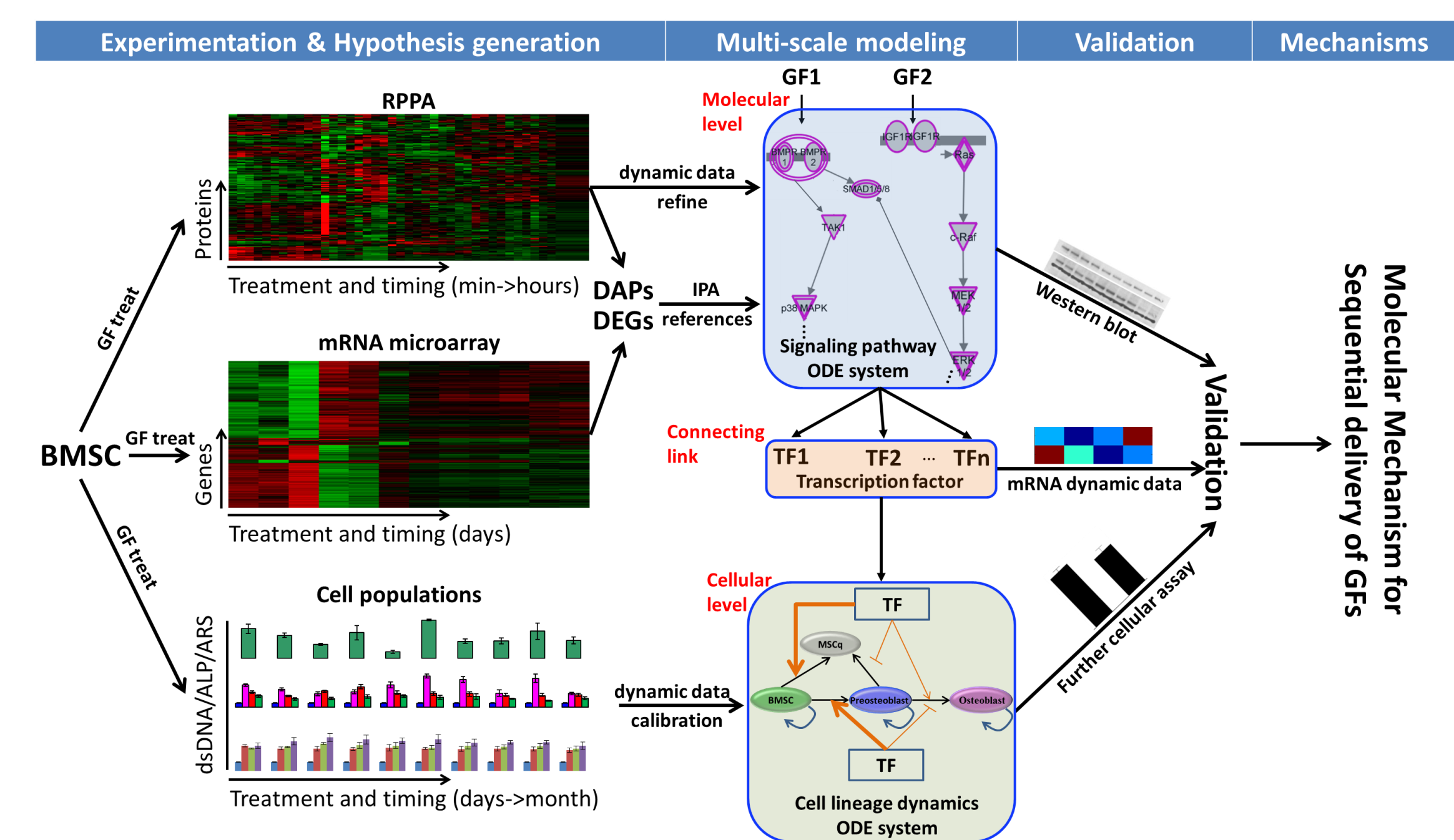


## Overall - Systems Biology Design

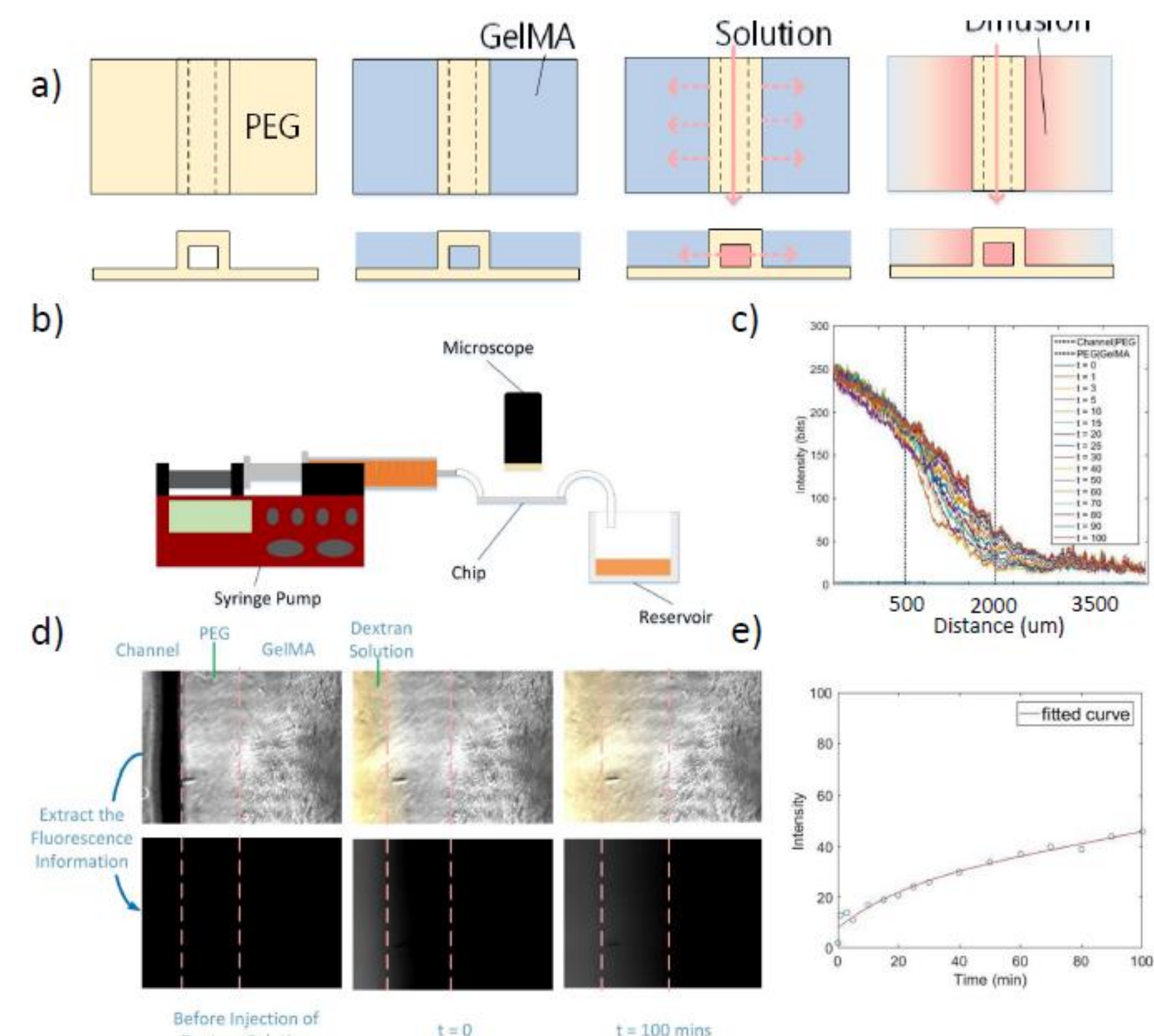
- We will first purify and implant the WJ-derived stem cells into the surgery-created cleft site in animal, with or without scaffold and histone modifier (HM) for comparison, and then measure the dynamic molecular and phenotypic changes at multiple time points.
- The protein levels of essential genes will be detected by reverse phase protein array (RPPA) technique; the gene expression and transcription factor binding profiles will be monitored by RNA-seq and ChIP-seq assays, respectively; the morphological changes will be recorded by panel CT imaging. These data will be adopted to investigate relevant biomarkers, key regulators, and critical signaling cascades.
- We will also construct a 3D multiscale model to study the tissue regeneration process by simulating the stem cell lineage commitment incorporating the molecular and cellular data.



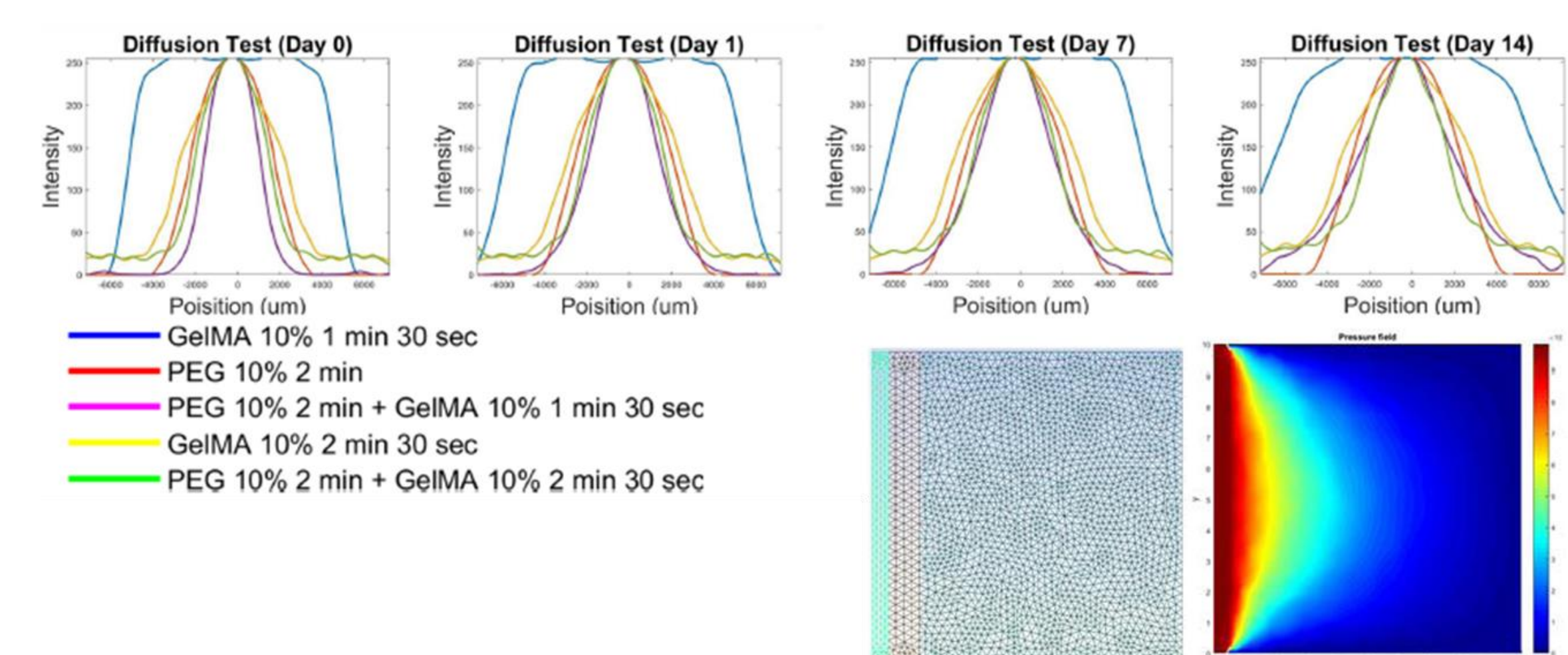
## Project progress – year 2



Systems biology approach to studying the molecular mechanisms for sequential delivery of growth factors.

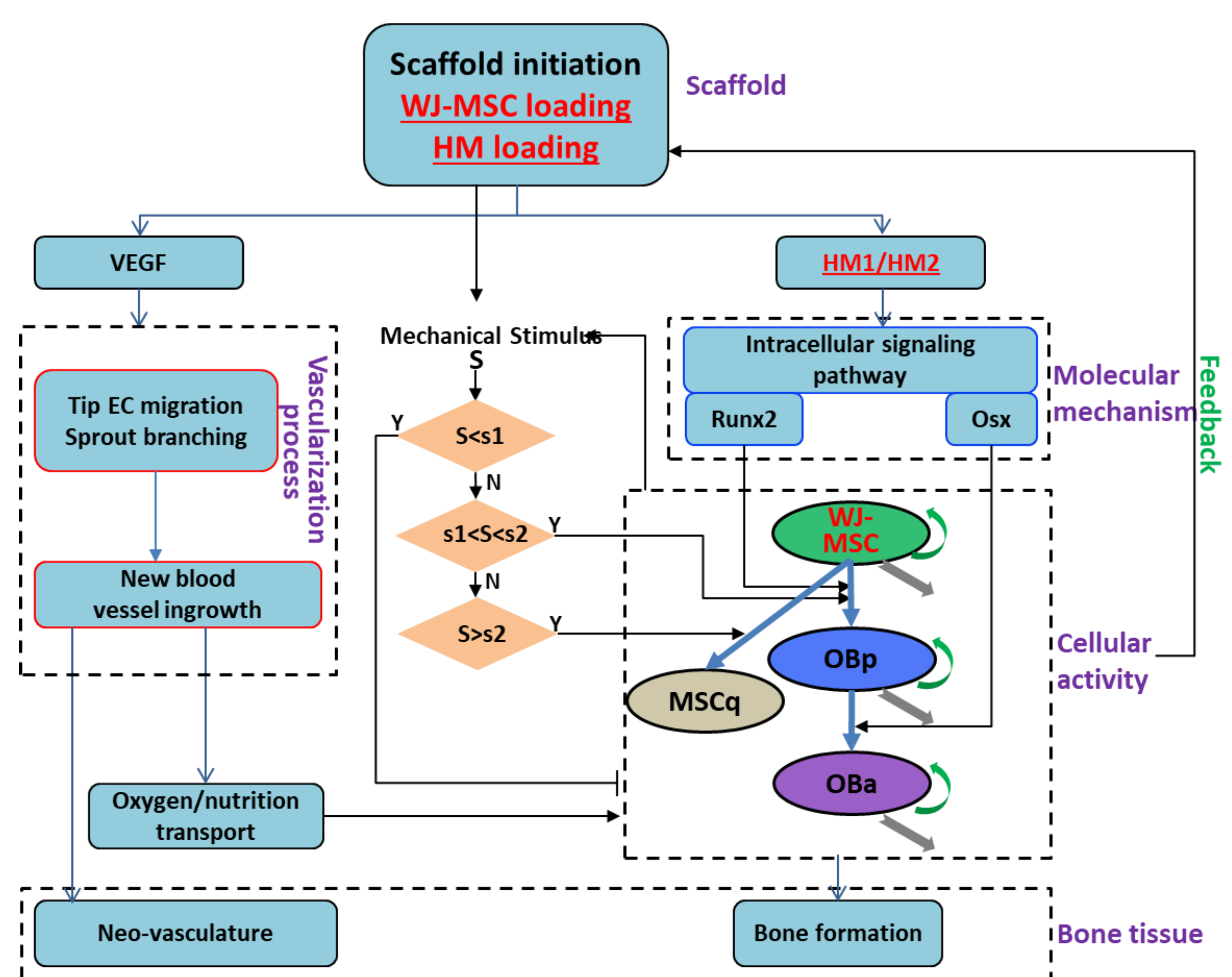


Device and experimental set up for cytokine diffusion test. (a) The process of manufacturing the diffusion device, which includes a PEG channel and a GelMA environment. (b) The experimental setup of the diffusion test. (c) The images captured by the microscope were further analyzed in MATLAB. (d) The bright field and the fluorescence images before the injection of dextran ( $t = 0$ -), right after the injection ( $t = 0+$ ), and after perusing 100 minutes. (e) The changing dextran intensity with time can be analyzed by the image processing functions in MATLAB.



Experimental and simulation results for cytokine diffusion in the dual-hydrogel system

## Implementation – Integration of wet-lab and in-silico studies



**Schematic overview of the 3D multiscale model of bone regeneration with WJ-derived stem cells and histone modifiers.** Multi-scales refer to molecular (signaling pathway), cellular (cell interaction and behavior), and tissue (bone formation). A histone modifier can be an inhibitor of a DNA methyl transferase or histone deacetylase. WJ-MSC: human Wharton's Jelly-derived mesenchymal stem cell; OBp: preosteoblast; OBa: (matured) osteoblast; MSCq: assumedly quiescent MSCs. HM: histone modifier.

Time points (week)	0	4	8	12
WJ Treated		3	3	3
Control	3	3	3	3

**Sample collection schedule.** We will need 3 mice for each group at each time point as designated. A total of 3 samples will be harvested from each mouse, one for RPPA assay, one for RNA-seq, and the other for ChIP-seq assay for particular transcriptional factors or histone markers. Our preliminary experiments showed that the efficiency of extracting RNAs and proteins from bone is relatively low. We will adopt the state-of-the-art strategies to maximize the RNA/protein production rate while simultaneously keep their stability as previously proposed.

## Acknowledgements

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