INCORPORATING QUALITY IN ENGINEERED TISSUES USING BOTTOM-UP NICHE ASSEMBLIES

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A major limitation in Tissue Engineering (TE) is the ability to control complexity within 3D engineered constructs. Diffusion limitations lead to the development of uncontrolled or even adverse environments leading to uncontrolled stem cell fate decision within the cultured tissue and cell death. Moreover the lack of control of the environment within these constructs makes the application of quality engineering principles such as quality by design (QbD) impossible. Length-scales chosen for the creation of in vitro tissues have not been chosen based on rational criteria and hence minimal success has been attained upon implantation. Recently bottom-up strategies have been introduced advocating the use of smaller tissue modules as building blocks for the formation of larger tissues prior to implantation.

In this work we first cultured seeded human progenitor cells on non-adherent agarose surfaces containing microwells at their bottom, trapping the seeded cells, allowed initial condensations to take place and the formation of controlled-size aggregates (Figure 1A). After chondrogenic differentiation 3D cartilage intermediate µ-tissues where formed, positive for alcian blue and safranin-o stains indication the presence of mature cartilaginous extracellular matrix. These cartilaginous µ-tissues were fused via self-assembly for 24 hrs in vitro into larger implants with a diameter of 4 mm and implanted subcutaneously in small animal models. As control we used implants formed by progenitor cells cultured in pellet format in the same media formulation as the µ-tissues and containing the same amount of cells as the bottom-up assembled implant. Even at this scale a bone organ was formed in vivo containing a cortex and a bone marrow compartment while the macro-pellet demonstrated a large fibrotic tissue domain within the implant (Figure 1B, C).

We believe that this is a first step in establishing a biomanufacruting pipe-line for the robust production of 3D implants/tissues for skeletal regeneration. Moreover the bone forming ability of the fused µ-tissues in their in vivo performance provides an unprecedented potency assay that could directly link the process environment to the critical quality attributes of the manufactured tissues. Although demonstrated for a skeletal application in this study this strategy could be applied for the manufacturing of a variety of tissues.

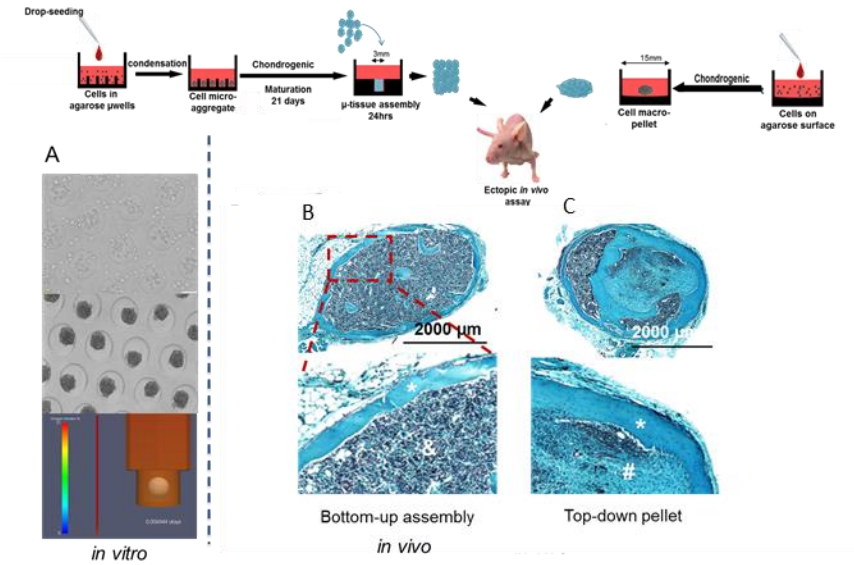

*Figure 1 – (A) images showing controlled size µ-tissues and model used to define their size, (B) Safranin-O stain of bottom-up assembled tissue, (C) Top down pellet culture explant. * denotes bone, & denotes bone marrow, # fibrotic tissue*