3D multi-layered cell-hydrogel tissue-like constructs

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Introduction: Tissue Engineering aims at the creation of functional three-dimensional (3D) tissues by the combination of different cell types, cell-instructive molecules, scaffolds or devices that facilitate cell differentiation and assembly into organized constructs. Recent strategies, in an attempt to mimic more closely the geometric complexity of biological tissues, move towards the control over the arrangement of biomolecules and cells in 3D. Microfabrication techniques, try to have control over the z direction following a layer-by-layer approach. Photolithographic, microfluidic, electrochemical pattering techniques, are used to create layers of materials that are patterned with adhesion ligands and/or living cells. By continuous deposition of these layers it is possible to form bigger 3D organized constructs. One of the main critics attributed to this layering approach is the loss of fidelity in the z dimension during the iterative procedures.

Cells in living tissues are surrounded by a viscoelastic macromolecular network which contains biological signals that determinate cell fate. They interact with their surrounding extracellular matrix (ECM) in a complex bi-directional way and have the ability to remodel it when necessary. In order to reconstruct a synthetic mimic of a naturally occurring microenvironment, we aim at giving the cells an initial position and providing them with physical as well as biological cues. This information will urge the cells to migrate, differentiate and reorganize the provisional tissue construct to form a tissue-like 3D construct. For that purpose, we propose the combination of PEG-based artificial extracellular matrices[1,2] together with 2D patterning techniques, followed by a layer-by-layer approach.

Materials and Methods:

PEG-hydrogel are formed by a factor XIIIa (FXIIIa) catalyzed cross-linking scheme, which forms isopeptide bridges between Gln and Lys residues of star shaped, peptide functionalized PEG macromers and results in a viscoelastic artificial matrix that is largely bio-inert. In order to make the system biologically responsive, peptide linker domains that are either sensitive or in-sensitive towards proteolytic digestion were chosen to form the matrix. Bioactive molecules such as growth factors (VEGF) or peptides (RGD) that are engineered to contain a XIIIa substrate sequences are covalently matrix tethered to the forming matrix, render it with defined biological properties.

Cell matrix suspensions were formed upon addition of FXIIIa and allowed to polymerize either as layers of homogenously 3D encapsulated cells or as precisely deposited spots in the x-y plane using a GMS 417 Protein Arrayer. Individual cell and / or matrix layers were assembled to form a highly ordered tissue-like constructs.

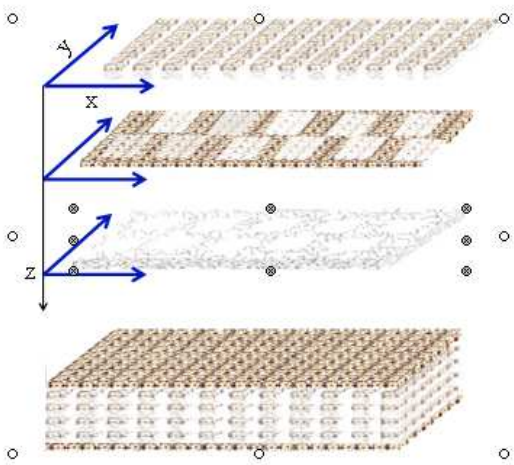

Fig. 1: Conceptual drawing of our approach.

Results and Discussion: Our results show that we are able to position viable cells in highly ordered 3D tissue constructs. As this constructs are built up by module-like layers of approximately 200 µm thickness the individual layers can be fabricated with spatially controlled features in the x-y plane. We show that either by control over cell instructive adhesion signals (e.g.: RGD) or by using different concentration of PEG we can direct cell spreading and migration of MC3T3 and C2C12 cells. We observe that cells do not migrate in absence of RGD or PEG concentrations exceeding 3%, but rather stay round. Thus, as cells cannot cope with this highly cross-linked and mechanically stable hydrogel networks or matrices that do not support cell adhesion, such conditions can serve as barriers that are not explored by migrating cells. Furthermore, we have shown recently[1,2] that growth factors can be tethered in a very predictable manner to fibrin-like matrices, which will enable specifically to direct the behavior of different cell lineages in a locally restricted area.

Conclusions: We believe that this approach is a powerful tool to control both distribution of cells and biological cues involved in tissue formation and a step further for the creation of 3D functional tissue-like constructs.

References: [1] Ehrbar M., *et al.*, Biomaterials, 2007. 28:p.3856-66. [2] Ehrbar M., *et al.*, Biomacromolecules, 2007. 8(10):p.3000-7.

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