Therapeutics Program (in the form of two large microarrays, each bearing 4,000 compounds in duplicate, on isocyanate-functionalized glass slide) against vascular endothelial growth factor (VEGF). We identified 170 ligands that bound VEGF with affinities in the range of 0.2 nM to 10 nM. By measuring binding curves of ligand-VEGF mixtures to surface-immobilized KDR with our scanners, we identified 12 compounds among the 170 ligands that actually inhibited VEGF-KDR reactions at compound concentrations below 100 μM. We further determined the IC50 values (albeit *in vitro* and microarray-based) for these 12 compounds ranging from 0.3 μM to 60 μM. The observed inhibition effect and IC50 values of these 12 compounds were confirmed by cell-based *in vivo* assays.

2118-Plat
3D Culture of Human Muscle Cells Modulates Cell-Matrix Adhesions and Actin Cytoskeleton Organization
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Anchorage of the muscular cells to the extracellular matrix is crucial for a range of fundamental biological processes including migration, survival and differentiation. However, muscle cell adhesions in a 3D environment and scaffold rigidity of engineering muscle tissue (EMT) remain to be determined. We aimed to characterize cell-matrix interactions in 3D muscle culture and to determine their consequences on cell fate. Human myoblasts were embedded in a fibrin matrix casted between 2 posts, cultured in 3D until confluence, then induced to differentiate. Microscopic analysis revealed that the formation of adhesion sites in 3D were smaller in size and number than in rigid 2D culture. The expression of adhesion site proteins, including α5 and αV integrins, vinculin and FAK, did not differ between 2D and 3D environments. Within the 3D myoblasts, the actin filaments typically formed cell projections and exhibited reduced actin stress fibers than their 2D counterparts. Concomitant to myotube formation in 3D, actin filaments became densely packed and displayed parallel aligned filaments oriented along the longest axis of the myotubes. Myoblasts and myotubes in 3D exhibited thicker and ellipsoid nuclei instead of the thin disk-like shape of the nuclei in 2D ($p<0.001$). Differentiation kinetics was faster in 3D as attested by higher mRNA expressions of α-actinin and myosin 1 day after differentiation switch. Importantly, the elastic modulus of EMT increased significantly from 3.5 ± 0.8 kPa to 7.4 ± 4.7 during proliferation and reached 12.2 ± 6.0 kPa during differentiation (each $p<0.05$), thus attesting to increased stiffness during myocyte differentiation. In conclusion, we reported modulations of adhesion, actin cytoskeleton and nucleus shape in 3D muscle culture. Therefore, our results pointed out complex interactions between muscle cells and the surrounding matrix with dynamic regulation of the cell-matrix stiffness.

2119-Plat
Active Micropatterns, New Tool for Single Cell Studies
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Surface micropatterning is a powerful tool for the design of cell-based assays (Quantitative Cell Phenotyping, Toxicology) and sensors, or for fundamental studies of cellular response to environmental cues. The combination of surface chemistry and microfabrication techniques allows to create substrates onto which adhesion can be tuned so as to obtain regular 2D arrays of immobilized cells. Such patterns have proven to be highly valuable for e.g. statistical analysis of the response of cells cultured in a well-controlled microenvironement (drug delivery assays). Many different strategies have been developed to fabricate surfaces presenting cell-adhesive patterns, among which the most popular are probably microcontact printing and photolithography. These techniques have advantages and drawbacks in terms of ease of use, reproducibility, large scale homogeneity of the patterns and stability of the produced surfaces. Here we present a new micropatterning technique dedicated to single cell studies: this technique leads to a fast and robust fabrication of high-resolution thermoresponsive micropatterns of which we think that the combination with microfluidic is a promising technology for drug or toxicology assays.

2120-Plat
Controlling Embryonic Cell Sheet Migration using Microfluidics
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Embryonic development consists of a complex series of cell signaling, cell migration and cell differentiation events whereas morphogenesis is the process that controls the organized spatiotemporal distributions of these events. Cell sheet migration is central to embryonic development, complex organs system and diseases, therefore studying cell migration within sheets is important. Embryos from the African Claw-toed frog, Xenopus laevis, are used to elucidate genes important in moving cells. However, little is known about the underlying mechanism by which cells in epithelial sheets coordinate their responses to growth factors, directional signals, and motility cues to direct sheet movement in vivo. One reason for this knowledge gap is the lack of technologies to control of the chemical microenvironment surrounding multicellular tissues. Here, we manipulate the chemical microenvironment with precise spatiotemporal delivery of cytoskeletal inhibitors and contraction-stimulating compounds using laminar flow interfaces with microfluidics. We use this approach to study motility of the cells within the sheets to these localized environments and understand how their coordinated behavior works with spatiotemporal control.
Microfluidics provides an opportunity to control chemical stimulation of biological systems using laminar flow interfaces with common flow modulation methods. To deliver precise chemical stimulation to a multicellular tissue, we have developed a system for controlling the inlet pressures to a microfluidic device by modulating fluidic resistance and capacitance. We employed this system to deliver chemicals over tissue explants from Xenopus embryos with a spatiotemporal control to study mechanical patterning and local control of cell sheet migration during epibolic-type morphogenetic movements.
We believe that patterning cell mechanics and controlling cell motility provide a means to initiate synthetic morphogenetic programs. In addition, the ability to control the form of multicellular tissues potentially has high impact in tissue engineering and regeneration applications in bioengineering and medicine.

Minisymposium: Ligand-gated Channels

2121-MiniSymp
A Prokaryotic Perspective on Pentameric Ligand-Gated Ion Channel Structure and Function
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The X-ray structures of two prokaryotic pentameric ligand-gated ion channels (pLGICs) have provided first structural insight into the family at high resolution. The structure of GLIC, a proton-activated channel from the cyanobacterium Gloebacter violaceous shows an open conformation of the pore. The channel conducts cations with similar properties as the nicotinic acetylcholine receptor and is inhibited by the same set of open channel blockers. The transmembrane pore is funnel-shaped with a wide hydrophobic entrance at the extracellular side that narrows to a hydrophilic intracellular opening. In this region conserved residues coordinate ions which have lost a large part of their hydration shell.
The structure of ELIC, a pLGIC from the plant pathogen Erwinia chrysanthemi shows a non-conducting conformation of the channel that was obtained in the absence of ligands. In its structure the extracellular half of the pore is occluded by bulky hydrophobic residues that likely prevent ion conduction. ELIC is activated by a set of primary amines that include the neurotransmitter GABA. The protein forms cation selective channels with large single channel conductance that slowly desensitize in the presence of ligands.
The strong structural similarity to their eukaryotic counterparts combined with their comparably simpler functional behavior make ELIC and GLIC important model systems for the pLGIC family that will ultimately allow a detailed comprehension of mechanistic properties that are still only poorly understood.