# Stem cell condensation and gap junctional communication on nanopatterned substrates for improved cartilage formation

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Mesenchymal condensation is a prevalent morphogenetic transition, regulated by cell adhesion, in which mesenchymal stem cells (MSCs) migrate towards each other. In osteochondral development, this is concurrent to the formation of an extensive gap junctional intercellular communication (GJIC) network. Little is known about the way the environment modulates the formation of this network and its implications in tissue architecture and function. We previously developed substrates nanocoated with the cell adhesive arginine-glycine-aspartic acid (RGD) peptide to tailor local surface adhesiveness at the nanoscale. Substrates were characterized with Atomic Force Microscopy, and local areas with a mean interparticle distance shorter than 70 nm were considered as adherent. We showed that substrate ligand density modulates the expression of chondrogenesis markers [1,2].
Here we studied the influence of local ligand density on mesenchymal condensation and the establishment of a functional GJIC network, by assessing expression and spatial disposition of GJ protein Cx43 and by a tracer intake assay in cell condensates. Substrates with a high percentage of cell-adherent area (90%) promote stable cell condensation, differentiation and Cx43 expression. Cx43 expressed in these condensates forms a tighter communication network than in other substrates of lower ligand density, as shown by the tracer intake assay. To understand the effect of ligand density on tissue formation after condensation, formed condensates were transplanted to new substrates of either the same or a different ligand density, and Cx43 expression was quantified. Transplantation of formed condensates to fresh optimal substrates further increased Cx43 expression, which does not occur in condensates transplanted to low ligand density substrates. We therefore conclude that nanoscale ligand density regulates not only the process of mesenchymal condensation, but also concurrent protein expression and its spatial disposition during differentiation, affecting the functionality of developing tissue [3].

## REFERENCES

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