TENSIOMETRIC ESTIMATION OF MATERIAL PROPERTIES OF TISSUE SPHEROIDS

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Tissue spheroids have been proposed to use as building blocks in biofabrication and as bioinks in 3D bioprinting technologies. Tissue fusion is an ubiqious phenomenon during embryonic development. Biomimetic tissue spheroid fusion is a fundamental constructional principle of emerging organ printing technology because closely placed tissue spheroids could fuse into tissue and organ-like constructs in fusion permissive bioprintable hydrogel. From physical point of view tissue spheroids could be considered as a visco-elastic-plastic soft matter or complex fluid. We hypothesize that quantitative estimation of material properties of tissue spheroids using tensiometry could predict their tissue spreading and tissue fusion behavior as well as provide a powerful insight about possible speed of post-printed tissue and organ-like constructs compaction and maturation.

Tissue spheroids from human fibroblasts, ovine and human chondrocytes and immortalised human keratinocytes have been biofabricated using non-adhesive cell culture plates (Corning, USA). For estimation of material properties of tissue spheroids commercial tensiometer Microsquisher have been emploied (CellScale, Toronto, Canada). Modulus of elasticity of tissue spheroids have been calculated based on peformed tissue compression tests. In order to identify structural determinants of material properties of tissue spheroids standard perturbants of cytoskeleton such as Cytochalasin D (Sigma, USA) for disruption of microfilaments and Nocodazole (Sigma, USA) for disruption of microtubules have been used. Viability of tissue spheroids have been also estimated and their morphology have been analysed using light microscopy, histochemistry, immunohistochemistry, semithin sections stained wih toluidine blue and transmission and scanning electron microscopy. Kinetics of tissue spheroids spreading on electrospun polyurethane matrices have been analysed. Kinetics of two closely placed tissue spheroids fusion have been analysed in hanging drop. Additionally toxic effect of water solution of paramagnetic gadolinium salt (Omniscan®, GE Health Care, USA) on material properties of tissue spheroids have been investigated.

It have been demonstrated that material properties of tissue spheroids biofabricated from different cell types have different modulus of elasticity. Even tissue spheroids biofabricated the same cell types but from different species have different material properties. Incubation with Cytochlasin D dramatically reduces estimated material properties of tissue spheroids. Incubation with Nocodazole does not significantly change material properties of tissue spheroids. Material properties of tissue spheroids from chondrocytes (chondrospheres) correlates very well with increasing deposition and accumulation of extracellular matrix (confirmed by expression of collagen type II and glycosoaminoglycans). The incubation with toxic concentration of gadolinium solution dramatically reduces material properties of chondrospheres. There is no any significant correlation between material properties of tissue spheriods and their spreading kinetics. However, there is a certain correction between material properties of tissue spheroids and their tissue fusion kinetics.

Our data demonstrate that beside already well established role of cell adhesion receptors such as cadherin and integrins in the realisation of cell cohesion inside tissue spheroids the structural determinants of material properties of tissue spheroids also include components of cytoskeleton such as actin micofilaments and accumulated extracellular matrix. It is possible to predict post-printing tissue fusion behaviour of tissue spheroids based on preliminary estimation of their material properties. Finally, it have been also shown that material properties of tissue spheroids correlate with their viability. Thus, tensiometry is a valuable method for systematic characterization of material properties of tissue spheroids and for prediction of tissue spheroids post-printed tissue fusion behaviour.