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Peripheral nerve injuries often result in sensory and motor dysfunction in respective parts of the body. So far, peripheral nerve regeneration is often associated with poor functional recovery. Important facilitators of the regeneration process are Schwann cells (SCs), which basement membrane is chiefly comprised of laminin. Extracellular vesicles (EVs) are considered important for intercellular communication and transfer of biological information. Mesenchymal stem cell-derived EVs (MSC-EVs) have been identified as a new therapeutic option due to their function as a drug delivery system. However, the precise delivery of EVs to the site of interest upon administration remains challenging. To overcome this issue, overexpressed EV surface marker protein CD81, from the tetraspanin protein family, has been modified toward preferential binding of laminin.

This study was designed to achieve production of laminin-binding EVs derived from MSC by modification of the large extracellular loop (LEL) of CD81.

Specific CD81-LEL sequences are cloned into lentiviral vectors encoding the expression cassette for full-length CD81 proteins fused with eGFP or firefly luciferase under the control of human cytomegalovirus (CMV) promoter. Stable cell lines are obtained upon transformation of Wharton's Jelly MSCs and selected by sorting for high expressers. MSC-EVs are further isolated by ultracentrifugation and characterized by nanoparticle tracking analysis, flow cytometry and western blot.

Our results demonstrate the feasibility of production of designed laminin-binding EVs derived from MCSs. This study represents the basis for further investigation on EVs regarding their targeted binding to laminin, their internalization by SCs and their influence on peripheral nerve regeneration processes.

Abstract 1971

DEVELOPMENT OF NEW GENERATION HYDROCOLLOID BIO-INK FOR 3D BIOPRINTING

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Bioprinting enables the production of 3-dimensional (3D) structures by combining bioinks, living cells, extracellular matrix (ECM) components, biochemical factors, proteins, drugs; and it has recently become one of the most promising techniques in the field of tissue engineering. The successful production of the 3D structure to be created by 3D bioprinting technology depends on the properties of the bio-ink to be used. Hydrogel/hydrocolloid materials used as bio-inks are developed using synthetic and natural polymers where they have the necessary rheological properties for printing, they also have biocompatibility, low toxicity and support for cell attachment. Natural hydrogels, which have the ability to mimic the extracellular matrix structure and function at a high rate, are highly preferred bioink materials for bioprinting applications. Polysaccharide-based hydrogel/hydrocolloids are one of the largest subclasses of natural polymers and are commonly used in food industry, drug release and tissue engineering applications with their gelling and biocompatibility properties. Hydrocolloids obtained from the seeds of some plants are among the promising natural materials in tissue engineering applications and the development of new generation bio-inks with their high water holding capacity, anti-inflammatory, and antioxidant properties. Here we report development of a new generation polysaccharide-based bio-ink for 3D bioprinting applications. The bioink was obtained through ''from waste to the bench-top'' approach by utilizing quince seed as a raw material. It was shown that developed bioink demonstrates desirable properties including viscoelasticity and processability, biocompatibility and non-toxicity, as well easy to obtain and cost effective as a bioink.

Keywords: 3D Bioprinting; polysaccharide bio-ink; 3D cell culture

References

1. Arslan-Yildiz, A., El Assal, R., Chen, P., Guven, S., Inci, F., & Demirci, U. (2016). Towards artificial tissue models: past, present, and future of 3D bioprinting. Biofabrication, 8(1), 014103. 2. Hospodiuk, M., Dey, M., Sosnoski, D., & Ozbolat, I. T. (2017). The bioink: A comprehensive review on bioprintable materials. Biotechnology advances, 35(2), 217-239. 3. Gungor-Ozkerim, P. S., Inci, I., Zhang, Y. S., Khademhosseini, A., & Dokmeci, M. R. (2018). Bioinks for 3D bioprinting: an overview. Biomaterials science, 6(5), 915-946.

Abstract 1972

T CELL ACTIVATION DIRECTS ARTICULAR REPAIR OF FULL-THICKNESS OSTEOCHONDRAL DEFECTS IN RAT

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Osteochondral defects in the adult human fail to heal, resulting osteoarthritis (OA). The onset of OA has been linked to a prolonged pro-inflammatory response induced by the injury or repetitive microtrauma. This may be caused by an imbalance in the signalling cascades during the transition from the pro-inflammatory to the pro-regenerative phase.

Small (Ø: 0.15mm, SD) or large (Ø: 1.5mm, LD) full-thickness osteochondral defects were created in the trochlear groove of 10-week old wild type (T-cell+) or T cell deficient (T-cell-) female rats. Healing was characterized up to twelve weeks. Pathological evaluation confirmed that the T-cell+-SD model displayed functional healing (OARSI score 0/4), T-cell+-LD resulted in moderate fibrosis (2/4) and Tcell–SD and Tcell–LD displayed mild (1/4) and severe (3/4) fibrosis, respectively. Analysis at one week confirmed a corresponding trend between the healing potential to extracellular matrix (ECM) production, progenitor- and inflammatory cell activation. Interestingly, scRNAseq confirmed a unique inflammatory-progenitor cell population present within the defect area in the Tcell+-SD model. Injection of in vitro cartilage-activated lymphocytes, placenta derived progenitor cells (PLCs) or a 24h co-cultured combination thereof 1 week post defect creation improved the healing. But only co-cultured cells completely regenerated the Tcell–SD and LD defects based on OARSI scoring and lubricin secretion.

These results confirm the integral role of the balanced activation of lymphocytes and progenitor cells for functional osteochondral regeneration. Furthermore, the presented findings show effective, stable articular cartilage regeneration after combined treatment of cartilage-activated lymphocytes and PLCs in moderate to large osteochondral defects.

Keywords: Regeneration; Osteoarthritis; Cell therapy

Abstract 1973

BIOFABRICATION BY MAGNETIC LEVITATIONAL ASSEMBLY OF CELLS INTO DEFINED 3D CELLULAR STRUCTURES

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In the field of tissue engineering 3D (three dimensional) cell culture studies have increased over the years since they are the closest models of real tissues. Compared to the 2D models, there is a big improvement on cell growth, morphology, differentiation, gene and protein expression when 3D system is utilized. Because of these advantages 3D cell culture is commonly used for tissue engineering, artificial organ technologies, regenerative medicine, drug development, drug screening and stem cell studies. Despite promising advances in these areas, there are still unmet needs to completely fulfill all requirements. Sophisticated tools, methodologies and materials are still required for further development in tissue engineering; especially for cellular assembly, single cell level control, easy control over biofabrication system, direct forward cellular imaging and analysis. Recently, magnetic levitation technology that overcomes most of the above mentioned problems, has been utilized for the formation of 3D cellular structures. Magnetic levitational assembly of cells provide rapid, simple, cost-effective 3D cell culture formation while ensuring scaffold-free microenvironment.

This contribution summarizes our efforts in designing new setups and assembling cellular entities via contactless magnetic manipulation as functional biological units for 3D cell culture and tissue engineering. It has been demonstrated that especially cell-cell interactions are favored by utilizing magnetic levitational assembly. With the developed technology, employed models also provide us possibility to adapt several components into different platforms for further tissue engineering applications.

Keywords: Magnetic Levitation; Cellular Assembly; 3D cell culture

References

1. Turker E, Arslan Yildiz A. Recent Advances in Magnetic Levitation: A Biological Approach from Diagnostics to Tissue Engineering. ACS Biomaterials Science and Engineering (2018) 4:787-799. 2. Turker E, Demircak N, Arslan Yildiz A. Scaffold-free cell culturing in three-dimension using magnetic levitation. Biomaterials Science (2018) 6:1745-1753. 3. Onbas R, Arslan Yildiz A. Fabrication of Tunable 3D Cellular Structures in High Volume Using Magnetic Levitation Guided Assembly. ACS Applied Bio Materials (2021).

Abstract 1974

DYNAMIC BIOFUNCTIONALIZATION AND MODULATION OF HYDROGELS BY PEPTIDE-FOLDING MEDIATED INTERACTIONS AND BIOORTHOGONAL CROSSLINKING FOR 4D BIOPRINTING

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3D bioprinting is a promising method for generating cell- and tissue models and constructs for drug development, tissue engineering, and fundamental studies of cell-matrix interactions. The properties of the bioinks, comprising biomaterials and cells, determine printability, cell viability, and post-printing cell survival and behavior. Most current hydrogel-based bioinks provide limited possibilities to tune and alter the generated structures' properties after printing. Here, we describe a novel hyaluronan/poly(ethylene glycol) based hydrogel system for bioprinting that combines robust and tunable covalent bioorthogonal cross-linking strategy [1] with peptide-folding mediated interactions to enable dynamic modulation of hydrogel properties [1,2]. The peptides were de novo designed to homo- or heterodimerize and fold into a helix-loop-helix motif and dimerize into four-helix bundles. Peptide dimerization enabled dynamic modulation of cross-linking density and hydrogel functionality before, during, and after printing[2]. A toolbox of functionalized peptides with different functionalities was developed, which allowed for altering cell adhesion and enhancing retention of biofunctional enzymes. The latter was demonstrated by triggering enzyme-mediated biomineralization of the printed structures [1]. This flexible strategy for controlling and changing hydrogel properties can facilitate the development of 4D bioprinting techniques.

References

1. Aronsson C, Jury M, Naeimipour S, Boroojeni FR, Christoffersson J, Lifwergren P, Mandenius CF, Selegård R and Aili D. Dynamic peptide-folding mediated biofunctionalization and modulation of hydrogels for 4D bioprinting. Biofabrication, 12(3), p.035031, 2020; 2.Selegård R, Aronsson C, Brommesson C, Dånmark S and Aili D. Folding driven self-assembly of a stimuli-responsive peptide-hyaluronan hybrid hydrogel. Scientific reports, 7(1), pp.1-9, 2017;

Abstract 1975

EVALUATION OF ELASTOMERIC POLYMERS FOR MYOBLAST SURVIVAL, PROLIFERATION AND DIFFERENTIATION

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For developing tissue-engineered skeletal muscle three-dimensional scaffolds may provide a physical support. Among a variety of biomaterials developed and used for soft tissue engineering, elastomeric scaffolds remain promising due to their compatibility with the elastic properties of the host tissue. Elasticity, which implies recovery from deformation, is of particular importance in the context of dynamic tissues like muscle. We evaluated porous elastomeric 3D scaffolds from the family of poly(polyol sebacate) for survival, proliferation and differentiation of murine and human myoblasts. This family of soft, biodegradable chemically cross-linked elastomers are biocompatible and inexpensive [1]. They have already been studied in cardiac [2], [3] and vascular tissue engineering (reviewed in [4]) but not yet in skeletal muscle tissue engineering.

Several compositions of elastomers were evaluated with alamar blue and live/dead assay for myoblast survival over 6 days and based on these results, PGS 1:1 was chosen for further differentiation analysis. Preliminary data on myoblast differentiation capacity when integrated in the PGS 1:1 scaffold was generated using human myoblasts. From confocal imaging performed after staining for the differentiation marker tropomyosin, we observed efficient differentiation into myofibers throughout the scaffold. PGS 1:1 is a suitable, biocompatible scaffold for skeletal muscle tissue engineering allowing for myofiber formation.

References

1. Li et al. RSCA Advances 2012. 2. Chen et al Biomaterials 2010. 3. Engelmayr et al. Nat Mater 2008. 4. Hiob et al. Curr Opin Biotechnol 2016.

Abstract 1977

INTRAVASCULAR INFUSIBLE EXTRACELLULAR MATRIX MODULATES INFLAMMATORY RESPONSE IN MOUSE MODEL OF SYSTEMIC INFLAMMATION

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