Abstract 112

EFFICACY OF PRECONDITIONED OR GENETICALLY MODIFIED IL4-SECRETING MESENCHYMAL STROMAL CELLS IN A MODEL OF STEROID-ASSOCIATED OSTEONECROSIS OF THE FEMORAL HEAD IN RABBITS

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INTRODUCTION: Bone healing can be augmented by preconditioning MSCs (pMSCs) with inflammatory cytokines. Another approach is timely resolution of inflammation using immunomodulatory cytokines. We investigated the efficacy of pMSC and genetically modified MSCs that over-express IL-4 (IL4-MSCs) on early stage steroid-associated osteonecrosis of the femoral head (ONFH) in rabbits.METHODS: 36 male mature NZW rabbits received methylprednisolone acetate (20mg/kgIM) 4 weeks before surgery. There were 6 groups: 1. Core Decompress (CD) alone – a 3 mm drill hole+ injection of:2. hydrogel (HG) - 200 µl of hydrogel carrier3. MSCs–1 million rabbit MSCs4. pMSC - LPS (20 µg/ml) + TNFα (20 ng/ml) preconditioned MSCs 5. IL4-MSCs – rabbit IL-4 over-expressing MSCs 6. IL4-pMSCs – preconditioned IL-4 over-expressing MSCsEight weeks after surgery, femurs were evaluated by microCT, biomechanical, and histological analyses.RESULTS: Bone mineral density (BMD) and bone volume fraction (BVF) increased outside the CD in the pMSC group compared to the CD and MSC groups ($p < 0.05$). IL4-pMSC group was increased compared to the CD group ($p < 0.05$). The percentage of empty lacunae in the IL4-MSC group was significantly less than other groups outside the CD ($p < 0.05$); however, IL4-MSC group had less trabecular bone formation inside the CD. DISCUSSION: pMSC increased new bone formation after CD in ONFH; IL4-MSCs decreased the number of empty lacunae. Immunomodulation of bone healing has the potential to improve bone healing after CD for early stage ONFH; these interventions must be applied in a temporally sensitive fashion.

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Abstract 113

APPLICATION OF SECRETOME FROM HYPOXIA-PRECONDITIONED MESENCHYMAL STEM CELLS ON CARTILAGE REGENERATION

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Mesenchymal stem cell (MSC) secretome-based therapy is regarded as a promising treatment for cartilage lesions due to the action of MSC secretory factors. Hypoxia, as the physiological condition of MSCs, increases its proliferation, survival and paracrine activity, and has been shown to enhance angiogenesis, migration and suppress inflammation in various disease models. In this study, we investigate the effect of conditioned medium (CM) generated from hypoxia-preconditioned human bone marrow MSCs on cartilage regeneration. MSCs were subjected to normoxic (20% O2) or hypoxic (1% O2) conditions to generate CM. Treatment with hypoxic CM enhanced the proliferation and migration of both MSCs and chondrocytes. Hypoxic CM exerted a superior effect over normoxic CM in the relief of IL-1β induced upregulation of pro-inflammatory genes (COX2, IL-6) and hypertrophic marker, COLX in chondrocytes exposed to IL-1β. In addition, extracellular matrix degrading enzyme, ADAMTS5 was significantly suppressed by hypoxic CM, resulting in the rescue of IL-1β induced degradation of extracellular matrix. Furthermore, protein array analysis revealed increased production of chemokine (SDF1), anti-inflammatory factors (IL1-ra and GDF15) and growth factors (VEGF, HGF and FGF2) in hypoxic CM relative to normoxic CM, correlating to the functional augmentation of hypoxic CM. Taken together, the results demonstrate that hypoxic condition was able to significantly potentiate the migration and proliferation capability, as well as the chondro-protective and anti-inflammatory paracrine function of MSC secretome. Hypoxia could be a more efficient culture platform to improve the repertoire of MSC secretome for cartilage regeneration.

Abstract 114

BIOMECHANICS OF BONE TISSUE REGENERATION IN 3D POROUS STRUCTURES: A MECHANO-DRIVEN APPROACH

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Large bone defects represent a clinical challenge for which regenerative therapies and tissue engineering strategies aim at offering treatment alternative to conventional replacement approaches by metallic implants (1). Materials currently used for bone tissue scaffold fabrication are inorganic materials (magnesium, titanium, ceramics) and natural or synthetic polymers (write examples of both polymers, e.g. PCL). Some of them possess an interesting characteristic, that is degradability. Additionally, 3D printing technologies provide porous scaffolds with designed shape, controlled chemistry and interconnected porosity, where bone will regenerate (2).

In the last decades a strong effort has been made to optimize scaffold designs by means of computational tools. because the final aim is to promote bone regeneration with mechanobiologically optimized scaffolds. In fact, computer techniques and mathematically based models are becoming very useful tools for material engineers and biologists to advance the understanding of the scaffold behaviour under different environments (3). Regeneration algorithms elucidated the relationship between the tissue being formed within the pores and the loading environment, as well as the mechanical benefits of a degrading scaffold during bone formation (4).

In this work, a mechano-driven model is presented which recapitulates the mechanics of 3D printed porous scaffolds (non-degradable and degradable) and predicts short- and long-term bone regeneration. The scaffold regenerative potential based on the biomechanical contributions of both the host and the scaffold itself is also evaluated. Using this computational model, we demonstrated that the mechanical stimulus is intrinsically associated with the regenerative response to bone scaffolds in terms of bone formation.

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Abstract 115

THE BEST OF BOTH WORLDS: IN VITRO EVALUATION OF COMBINING TWO STEM CELL TYPES FOR TRUE CARDIAC REPAIR

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Myocardial infarction (MI) irreversibly destroys millions of cardiomyocytes in the ventricle, making it the leading cause of heart failure worldwide. Current therapies are unable to replace the lost cardiac tissue, thereby not preventing progression towards heart failure. As a novel regenerative therapy, a new cardiac stem cell type with high cardiomyogenic differentiation capacity, the cardiac atrial appendage stem cell (CASC), was identified. Although CASCs can improve cardiac function after MI, the application potential of CASCs is limited by the low cell engraftment and survival upon transplantation, largely due to their limited angiogenic properties. Since dental pulp stem cells (DPSCs) are highly angiogenic but lack cardiomyogenic properties, they can be used as a priming strategy to improve the efficacy of regenerative CASC therapy. This study investigated whether DPSCs are able to enhance CASCs properties in vitro by examining the effect on CASCs proliferation, viability, migration and angiogenesis. Rat CASCs were either cultured in conditioned medium of rat DPSCs or indirectly co-cultured with rat DPSCs. The effect of DPSCs on CASCs proliferation and viability was assessed by a propidium iodide assay in presence of 2% serum or serum-free medium respectively. Migration of CASCs was examined using a transwell migration assay. To investigate the effect on CASCs paracrine angiogenic properties, conditioned medium of treated and untreated CASCs was collected for in vitro functional assays using a human umbilical vein endothelial cell line (HUVEC). Endothelial cell proliferation, migration and tube formation were assessed. In ovo angiogenesis was examined using the chorioallantoic membrane assay.

Keywords: Preconditioning; Cardiac stem cells; Dental pulp stem cells

Abstract 117

TARGETING EARLY HEALING PHASE WITH TITANIA NANOTUBE ARRAYS ON TUNABLE DIAMETERS TO ACCELERATE BONE REGENERATION AND OSSEOINTEGRATION

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Blood coagulation and inflammation are the earliest biological responses to implant surfaces. It is well known that implant nanosurfaces can significantly impact the osteogenesis and osseointegration, which is most likely through the influence on the early phase of bone regeneration. However, the interplay between blood clot property and inflammatory reaction on nanosurfaces is less understood. This study investigated the influence of distinct surface properties of titanium on blood clot features, and whether the adaptable clot features are capable of steering osteoimmunomodulation targeting osteointegration. Titania nanotube arrays (TNAs) with different diameter were fabricated and in vitro evaluation with the whole blood indicated that TNA with a diameter of 15 nm (TNA-15) enabled noteworthy platelet activation resulting in distinct clot features compared with that of pure Ti and TNA with a diameter of 120 nm (TNA-120). The co-culture of macrophages (MΦs) with the clot showed that the clot on TNA-15 downregulated the inflammation and manipulated a favorable osteoimmunomodulatory environment for osteogenesis. In the animal model, TNA-15 downregulated inflammation-related markers (IL-1β, TNF, and IL8), while upregulated growth metabolism-related gene expression (BMP-2, WNT5A, and ITG-β1) in early healing hematoma identified by RNA sequencing. Eight weeks post-implantation in rabbit model TNA-15 promoted the de novo bone formation with improved extending of osteocyte dendrites, demonstrating the desired osseointegration. The results indicate that surface nano-dimensions can significantly influence blood clot formation, which manipulates a favorable osteoimmunomodulatory environment for bone regeneration and osseointegration.

Abstract 116

GUIDED TISSUE ASSEMBLY AND BIOFABRICATION OF MACRO-SCALED EPITHELIAL ORGANOID TUBES

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Epithelial organoids cultured in appropriate 3D conditions typically develop a micro-scaled, cystic structure lined with a polarized epithelium. Despite their great potential in research and therapy, epithelial organoids grow in heterogeneous sizes, and are too small to display physiologically relevant performance and applications. Here, we show guided assembly of mouse tracheal basal stem cell organoids towards geometrically-defined, lumenized constructs. The observed shape stability of organoid assembly is confirmed by theoretical modelling based on organoid morphology and the physical forces involved in fusion. We provide hypothesis on how epithelial organoid assembly can be achieved in a more efficient and predictable manner, of which principles could be extended to other organoid types developed form epithelial stem cells. The guided self-assembly strategy presented here opens up the possibility for biofabricating size-relevant, geometrically defined epithelial structures towards broad applications in biomimetic organoid-on-a-chip, and tissue engineering.

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Abstract 118

MODELLING OF CELL INVASION OF SCAFFOLD AT THE INITIAL STAGE OF SEEDING

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Cell seeding process influence the final tissue formation [1,2]. Preceding all other steps of tissue engineering, better cell adhesion and even spatial distribution are associated with improved culture results [3]. For understanding cell seeding process deeply, two geometry scaffold were designed to investigate the cell attachment process through experiment and simulation analysis, including a cubic design and a truncated octahedron design . A novel numerical model is developed by coupling the volume of fluid (VOF), discrete phase model (DPM) and cell impingement model (CIM) for predicting cell distribution after cell seeding. This methodology could help to predict initial stage of cell attachment clinical test more accuracy and also reduce a number of in vivo experiments.This method is able to predict the cell distribution and assessing the scaffold design. Truncated octahedron scaffold showed a more even distribution than cubic design in vitro cell seeding. Truncated octahedron scaffold with spatial distribution beams could provide a more suitble environment for nurtients transport and cell movement and distribution.

Keywords: Cell seeding; Computational Fluid Dynamics; Osteochondral scaffold