Towards the knee on a chip: development of a microfluidic platform for the mechanical stimulation of three dimensional cartilaginous constructs

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Extract from the Supervisor's reference letter

… Osteoarthritis (OA) is currently the most prevalent musculoskeletal disease. Even if it was estimated [1] that 60% of people aged 55 and older have radiographic evidence of OA, no satisfactory treatment is currently available for the pathology and its origin is still not clear. In this framework, the generation of adequate models both to study the basic mechanisms underlying the disease and to evaluate possible pharmacological or cellular therapies is a great need.

A proper mechanical stimulation, in particular, is instrumental in properly replicating the environment of human joints. For this reason, this master thesis dealt with the design, validation and biological exploitation of a microfluidic platform to mechanically stimulate cartilaginous constructs. Microfluidics, with its intrinsic advantages allows in fact an unprecedented control over experimental condition thus permitting the creation of the so-called Organs on a chip. Building on an existing patented technology used to provide cardiac cells with biaxial strains, a microfluidic device was designed to provide chondrocytes, encased in a novel PEG gel both cross-linked and biodegraded through enzymatic reactions, with a controlled state of confined compression either physiological (10%) or hyper-physiological (30%). The platform consists in three layers: a top layer, an actuation membrane and an actuation chamber. The top layer is made of a central channel containing the 3D cell laden hydrogel and two side channels for the culture media. The three channels are separated by two rows of overhanging pillars designed to properly confine the gel both during injection, preventing leakage in the side channels, and upon compression. The gap between overhanging pillars and the actuation membrane provides the compression level. An increased pressure in the actuation chamber indeed causes the actuation membrane to bend upwards until it reaches the mechanical stop provided by the pillars. Both analytical and computational methods were adopted in the design phase. In particular, Abaqus standard was used introducing a finite element model of the device to predict the effective strain field upon compression. A biphasic poroelastic constitutive relation was adopted for the cell-laden hydrogel, a Mooney Rivlin description was used for the hyperelastic PDMS. After computational validation and design, the device was subsequently fabricated through soft lithography and micro-molding and functionally validated checking the accuracy of the fabrication process and adherences of the performances to design criteria. The biological validation comprised an optimization of PEG gel formulation to achieve an optimal trade-off between gel stability and behavior in terms of cell proliferation and degradation rate. The device was subsequently exploited to i) generate a micro-cartilaginous tissue form human primary nasal chondrocytes and to subsequently ii) investigate its response to physiological and hyper-physiological compression. Upon 14 days of culture within the device under static condition, a shift towards a cartilage-like phenotype was observed. This was demonstrated by an increase in Glycosamminoglycans both accumulated within the constructs and released in the medium, and by a high deposition of Aggrecan and Collagen 2, proteins characterizing articular cartilage. Rt-qPCR also suggested an increase in COL2A1 expression over time up to 21 days of static culture. Subsequently the device was adopted to apply the over-cited levels of mechanical compression, namely 10% and 30%. After 14 days of static culture, stimulation was applied to the mature constructs, in two cycles of 2 hours separated by a 4 hour of break for 7 consecutive days. A custom-made control system was realized for the purpose. Compression applied after a period of static culture of 14 days caused a decrease in COL1A1 expression, proper of undifferentiated fibrocartilage, if applied at the 10% level and a statistically significant increase in MMP13 expression if applied at the hyperphysiological 30% level. Notably MMP13 is a collagenase involved in the matrix degradation proper of OA. In conclusion, a novel microfluidic platform, able to provide cells with a 3D culture environment and customized level of mechanical compression was designed and validated. The platform was successfully adopted in a preliminary assessment of the level-dependent effect of compression on cartilaginous micro- constructs, providing a first step towards the creation of a knee on a chip. …

ISBN: 978-88-6296-000-7