Intervertebral Disc Regeneration: Rheological and Biological Testing of a Novel Clinically-Relevant Hydrogel with MSCs

BACKGROUND: Back pain (BP) is strongly associated with intervertebral disc degeneration (IDD). Currently, there are no treatment options able to reverse the degenerative process targeting the pathophysiology involved in IDD. However, there is a strong effort to develop an effective early treatment of BP that may prevent, slow down, or reverse the degenerative changes in the intervertebral disc (IVD). Recovering the ability of the disc to repair the extracellular matrix and re-establishing the proteoglycan content may have a significant therapeutic effect by increasing disc hydration and thereby improving its biomechanics. Adult stem cell therapy may be considered a powerful tool in the future treatment of IDD. However, many open questions remain in the translation of this new cell therapy in the medical arsenal, such as the most reliable transplantation method including the surgical route to approach the disc, the carrier choice and the optimal cell dose. Moreover, development and rheological evaluation of injectable biomaterials as cell carriers that actively undergo gelation into the nucleus polposus (NP) is crucial to develop an effective stem cell-based approach. Functionalized hydrogels are ideal carrier systems for IVD regeneration since growth factors (GFs) may increase cell engraftment, stem cell differentiation and trophism.

AIMS: ***Aim 1:*** to study rheological properties of a new clinically relevant injectable hydrogel functionalized with autologous GFs from platelet rich plasma (PRP) blended with hyaluronic acid (HA) and a gelling agent, to generate an ideal carrier to deliver mesenchimal stem cells (MSCs) for IVD regeneration; ***Aim 2:*** to evaluate cell viability and the process of chondrogenic differentiation of human MSCs seeded into a clinical relevant hydrogel associated to PRP as GFs source.

MATERIALS AND METHODS: ***Aim 1 - Viscoelastic scaffold assembly and evaluation:*** PRP and HA blends (PRP/HA) were obtained at several compositions, to be used as a viscoelastic carrier for MSCs injection into the NP of the IVD. Batroxobin was used as the gelling agent, in order to obtain a 3D hydrogel system encapsulating MSCs in the presence of autologous GFs. Rheological properties were assessed by dynamic rheological measurements on the obtained gels using Anton Paar Rheometer MCR 502. Viscoelastic properties, along with the effects of different concentration of HA and PRP within the formulation, temperature effects and crosslinking were evaluated. Amplitude sweep, frequency sweep and flow curve were performed. The hydrogel composition (HA/PRP ratio) was chosen based on the outcome. ***Aim 2 – Evaluation of the effects of the bioactive PRP/HA hydrogel on hMSCs****:* Human MSCs were achieved from fresh bone marrow samples. hMSCs were suspended in human PRP to have a final cell concentration of 5x106 cells/mL obtaining the component A. 1.6% HA was mixed with BTX obtaining the component B. Hydrogels were obtained coupling components A and B. 100ul of the construct MSCs/HA/PRP/BTX were delivered in a 15ml tube and left to polymerize for 30 minutes, then growing media was added. After 24 hours, three types of culture media were used to culture the hydrogel: α-MEM with 10% fetal bovine serum, hMSCs differentiation basal medium-chondrogenic with 10 ng/mL Transforming Growth Factor- β1 and standard chondrogenic medium without growth factors. hMSCs seeded PRP-HA-BTX hydrogel were harvested at 7, 14 and 21 days. Cell proliferation and chondrogenic differentiation was evaluated by histological analysis, GAGs and gene expression.

RESULTS: ***Aim 1:*** 1,6% HA and BTX are the most suitable agents for the intended use. Gelation time of HA+PRP at 20°C is 15 minutes. It drops to 3 minutes in HA+PRP at 37°C. For pure PRP at 37°C the gel state was achieved in less than one minute. For the pure PRP gel the elastic modulus is circa 0.1kPa almost independent of the angular frequency. The composite gel including HA is featured by significantly lower storage modulus, and the difference between the storage and loss modulus decreased at higher frequency, almost reaching a crossing point at the angular frequency of 100 rad/sec. Moreover, BTX leads to a fast and reproducible gelation and a slower release of GFs since it doesn't act directly on platelets. ***Aim 2:*** hMSCs showed high metabolic activity demonstrating that the cells proliferate in this hydrogel composition. The cells cultured in chondrogenic condition with TGF-b showed an higher proliferation rate. Constructs cultured under chondrogenic condition with or without TGF-b showed a significant higher GAG production compared to control media. Human MSCs embedded in the PRP/HA/BTX hydrogel underwent differentiation in chondrocytes like cell under chondrogenic condition with TGF-b, as shown by the high metachromatic staining of Alchian Blue and the red staining with Safranin O compared to control and chondrogenic media without TGF-b. The gene expression levels of SOX-9, collagen type II and aggrecan increased significantly in TGF-ß group as compared to the control group and chondrogenic media w/o TGFb. Moreover, data suggest that MSCs in hydrogel expressed elevated levels of proteoglycans and chondrogenic genes expression compared to the control group.

ORIGINALITY OF THE PROPOSAL PROJECT: Starting from a set of technologies currently available and used for the treatment of degenerative and traumatic diseases of the musculoskeletal system, the project focused on the development of a **new biological tool based on *in vitro* expanded autologous bone marrow MSCs, autologous growth factors from PRP, and injectable HA**. This study demonstrated as a clinical relevant hydrogel composed by HA/PRP/BTX could improve the viability and proliferation of MSCs and above all promotes and supports the differentiation process in chondrogenic cells. Ex vivo and in vivo experimental procedures to test this hydrogel plus MSCs for IVD regeneration is in progress in an organ culture system using a bioreactor and an animal models, which may open the way to the translation in humans.