hepatocyte differentiation was induced for 28 days (D28). Two plating strategies were adopted to form the definitive endoderm: iPSC-monolayers (M) and iPSC-spheroids (S). To induce definitive endoderm, activin A and CHIR99021 were added to both plating conditions for 5 days. Prior hepatic maturation, iPSC-monolayers were also aggregated to form spheroids (MS). Subsequently, HGF and SB431542 were added to MS and S medium for 23 days. Spheroids were evaluated by real time PCR and immunofluorescence for hepatocyte-specific markers at D28.

Results: At protein level, the iPSC showed a pluripotent character through the nuclear presence of OCT3/4 and NANOG. At the end of the differentiation protocol, S differentiation strategy exhibited contractile cells. Albumin and cardiac troponin T were detected into the same spheroid by immunofluorescence. The MS strategy showed no beating cells. Also, CYP3A4 and albumin were detected by immunofluorescence and detectable mRNA levels were expressed for HNF4α, transferrin and alpha-1 antitrypsin.

Conclusions: Hepatocyte differentiation using spheroids requires a monolayer endoderm formation step in order to avoid the presence contractile cells at the end of protocol. Moreover, spheroid cells exhibited a great mature hepatocyte phenotype and can be used as a powerful tool for cell therapy, liver bioengineering, studies of drug efficacy and toxicity assays.

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THE CONNECTION BETWEEN AUTISM SPECTRUM DISORDER AND THE SCN2A AND RENL GENES

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Background: The incidence of autistic spectrum disorder (ASD) is growing, but the pathophysiology and the etiology is still uncertain. This disorder is caused by genetic and environmental factors causality, with the SCN2A and RELN genes being the most prominent associated to ASD. Hence, this study aims to investigate the role of SCN2A and RELN genes at ASD phenotype expression.

Methods: Previously produced induced pluripotent stem cells (iPS) lineages from healthy donors (EA1, EB4), SCN2A gene knockout (EB4CRISPR) and autistic patient (iM5) with this mutation proceed to cerebral organoids and neurospheres generation. Following maturation, their immunofluorescence analysis were performed. For statistical assay were used One-way ANOVA test with Tukey's post-test for multiple comparisons.

Results: iM5 embryoid bodies didn't develop cerebral organoids, in contrast to EB4 and EA1. All NSCs were cell-type validated with Q-PCR, indicating higher iM5 differentiation. iM5 neurospheres had atypical morphology and smaller neuronal extensions when compared with others at immunofluorescence. Also, knockout clone kept migrating and growing more than iM5 one – this clone also had a mutation at genes like RELN. At last, iM5 cultivated with conditioned medium (50% neurospheres basal medium and 50% medium collected from other neurospheres) from knockout clone showed better growing and migration, compared with healthy cells conditioned or basal mediums iM5 cultures.

Conclusion: Normal migration of knockout clone when compared with impaired one from iM5, improoved with conditionated medium, indicates that SCN2A doesn't have a main role at neuronal migration and leads to hypothesys that RELN is related to neuronal migration and growth. More experiments are needed to confirm this results.

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THE FREQUENCY OF PDPN+ CD146- HUMAN SKELETAL STEM CELLS AND ITS PROGENY VARIES IN ADULT BONES OF DIFFERENT ANATOMICAL LOCATIONS

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Background: Adult human skeletal stem and progenitor cells (hSSPCs), also known as bone marrow mesenchymal stem/stromal cells, are envisioned for bone bioengineering applications. Nevertheless, optimization of the procedures for the manufacture of clinical-grade cellular products are still needed. Recently, new phenotypic markers of the different hSSPCs subsets were described, but the frequency of these populations in bones of different anatomical locations are still unknown. In order to determine the best sourcing bone for improved hSSPCs isolation, in this study we evaluated the frequency of four cellular subpopulations within this system: the multipotent self-renewing skeletal stem cell pool (SSCs, PDPN+ CD146- CD164+ CD73+), the early bone, cartilage, and stroma progenitor (BCSPs, PDPN+ CD146+), the committed osteoprogenitors (OPs, PDPN- CD146+), and the chondroprogenitors (CPs, PDPN+ CD146-) in bone samples from hip and humerus.

Methods: Following ethical approval (CAAE n° 21768719.0.0000.5257), surgical waste bone from humerus or hip (acetabulum) were collected from patients of both sex, older than 18 years-old, undergoing primary arthroplasty. Following bone marrow dissociation and lineage depletion, the frequency of SSCs, BCSPs, OPs, and CPs in each sample was determined by FACS.

Results: In hip samples (n = 8), all four subpopulations were detected. SSCs accounted for 0.076% ± 0.20% of the CD45- CD31- pool, while BCSPs were 2.95% ± 3.95%, CPs were 8.29% ± 11.60%, and OPs were 7.41% ± 11.88%. In humerus (n = 3), however, only BCSPs and OPs were identified. Comparatively, the frequency of BCSPs in humerus was lower than in hip (0,025% ± 0,11%). In compensation, humerus samples had an increased frequency of OPs (25,05% ± 5,30%).

Conclusion: Our preliminary findings indicate that the frequency of the cell populations that compose the bone marrow SSPCs system significantly varies in bones, depending on their anatomical location. Therefore, the distinct bone sources investigated herein could be differentially explored to manufacture cellular products with specific combinations of SSPCs subsets and, consequently, with specific and more predictable biological properties. These tailor-made cellular products could translate in enhanced effectiveness in unique therapeutic contexts, changing the current perspective of trial-and-error in SSPCs application in skeletal regenerative medicine.

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THE IMMUNOMODULATORY EFFECT OF UMBILICAL CORD MESENCHYMAL STEM CELLS PRODUCED UNDER XENO-FREE CONDITIONS

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