1, 5, 10, or 20 g/L glucose and hMSC-free hydrogels loaded with 20 g/L glucose, which were implanted ectopically in nude mice. The newly-formed blood vessels at 21 days post-implantation was quantified using micro-CT scanner after microfil® injection.

Results: CM collected from hMSCs cultured with 1 or 5 g/L glucose promoted significant ($p < 0.05$) increased HUVECs migration when compared to the one collected from hMSCs cultured without glucose. hMSCs cultured in the presence of glucose released significantly ($p < 0.05$) higher amounts of Angiogenin, VEGF-A, a VEGF-C, Angiopoietin-1, Endostatin, and CCL2 when compared to hMSCs cultured without glucose. Most importantly, implanted hMSC-containing hydrogels loaded with either 5, 10, and 20 g/L glucose exhibited a 2.4-, 2.8-, and 2.4-fold increase ($p < 0.05$) in the volume of newly-formed blood vessels when compared to hMSC-containing hydrogels without glucose, respectively($n=8$). The volume of newly-formed blood vessels in cell-containing hydrogels without glucose and in cell-free hydrogels loaded with glucose were similar and minimal.

Conclusion: These data demonstrate that glucose promotes hMSC paracrine functions pertinent to angiogenesis.

Keywords: MSC, glucose, angiogenesis

doi:10.1016/j.bonr.2020.100372

P022

Towards in situ monitoring of *in vitro* 3D bone models

Donata Iandolo[a], Mikhael Hadida[a], Guénaëlle Bouët Chalon[a], Róisín M. Owens[b], Laurence Vico[a], David Marchat[a]
[a]*SAINBIOSE, INSERM U1059, Saint-Priest-en-Jarez, France*
[b]*Department of Chemical Engineering and Biotechnology, University of Cambridge, Cambridge, United Kingdom*

Cells are sensitive to numerous chemical and physical cues, imparted by both the fluid they are living in (*e.g.*, pH, glucose, O_2 and CO_2 concentration, flow speed and distribution, shear stress, hydrostatic pressure, reactive species), and the surface they are cultivated on (*e.g.*, surface chemistry and stiffness, wettability, micro and macro-architecture, release of chemical agents). Trying to recapitulate a physiologically relevant environment and control its parameters is thus a complex and fundamentally interdisciplinary challenge. We will develop a fully integrated platform comprising a perfusion system and purposely designed bioceramic scaffolds as they will guarantee to control the investigated fluidic environment with a high degree of confidence. This platform will allow us to carry out an extensive screening of cell responses to a range of reproducible mechanical and chemical environments and to suggest a map of the relationship of the mechanical environment and the induced cell response. The key innovative aspect of this project is the integration of innovative bioelectronics sensors within the perfusion system that will allow the continuous monitoring of cell proliferation and differentiation, leading to key insights in the underlying mechanism. A temporal profile of the secretion of the target analytes will be drawn and will be a relevant outcome of the project.

doi:10.1016/j.bonr.2020.100373

P024

Mesenchymal stem cells overexpressing BMP9 through CRISPR-Cas9 activation increase bone formation in rat calvarial defects

Gileade Freitas[a], Helena Lopes[a], Alann Souza[a], Isabella Santos[a], Adriana Almeida[a], Coralee Tye[b], Jane Lian[b], Janet Stein[b], Gary Stein[b], Marcio Beloti[a], Adalberto Rosa[a]
[a]*Bone Research Lab, University of São Paulo - School of Dentistry of Ribeirão Preto, Ribeirão Preto, Brazil*
[b]*The University of Vermont, Burlington, United States*

Cell therapy with mesenchymal stem cells (MSCs) induces bone formation in critical-size defects without resulting in complete regeneration. Cell therapy based on genetically edited MSCs is still underexplored. In this study we evaluated the in vivo effects of MSCs genetically edited by the CRISPR/Cas9 technique to overexpress BMP9. Mouse bone marrow MSCs were edited by lentiviral transduction with a dCas9-VPR-Puro vector and single guide RNA to target the BMP9 gene ($MSCs^{BMP9+}$). Cell editing was confirmed by increased gene expression of BMP9 and its targets Alk2, BMPR1 and 2, Hey1 and Dlx5. Calvarial defects 5mm in diameter were created in rats, and after 2 weeks the defects were injected with $MSCs^{BMP9+}$ or MSCs ($5x10^6$ cells in 50mL of PBS/defect), under approval of the Local Committee of Ethics in Animal Research. Four weeks post-injection, the newly formed bone was evaluated by micro-tomography (micro-CT) and the data were compared by Student's t-test ($p< 0.05$, $n=12$). The morphometric parameters generated from 3D-reconstructed micro-CT images indicated that bone volume, percentage of bone volume, bone surface and trabecular number were all higher in defects injected with $MSCs^{BMP9+}$ compared with control MSCs, while trabecular thickness and trabecular separation were lower (Fig. 1). These results indicate that MSCs with CRISPR/Cas9-mediated BMP9 overexpression increase bone repair. Our findings open new therapeutic possibilities for the treatment of bone defects based on genetically edited cells.

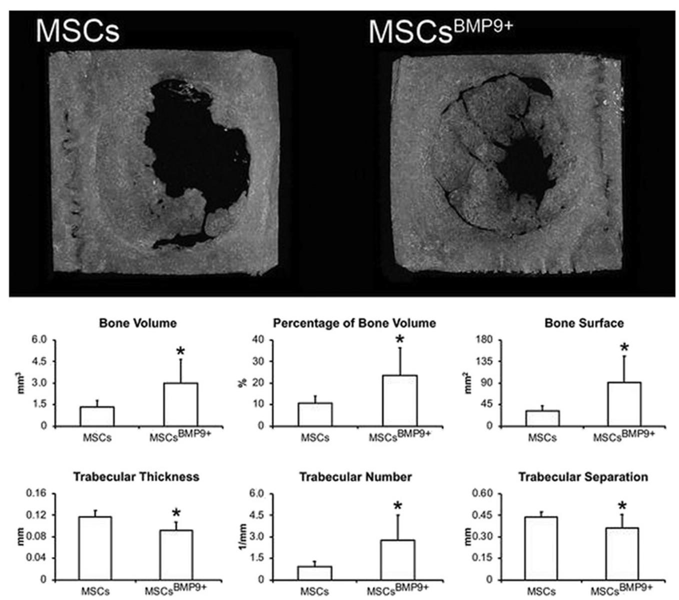

Fig. 1. 3D images and morphometry of bone tissue induced by MSCsBMP9+ and MSCs. * p<0.05.

doi:10.1016/j.bonr.2020.100374

P025

3D-printed cobalt-chromium porous metal implants showed enhanced bone-implant interface and bone in-growth in a rabbit epiphyseal bone defect model

Yu Ning Chim[a], Simon Kwoon Ho Chow[a], Sze Yi Mak[b], Michelle Meng Chen Li[a], Bob Ching Hang Yung[b], Edmond Wing Fung Yau[b], Elvis Chun Sing Chui[a]
[a]*The Chinese University of Hong Kong, Hong Kong, China*
[b]*Koln 3D Technology (Medical) Limited, Hong Kong, China*

Introduction: Cobalt-chromium(CoCr) alloys has the advantage over titanium implants in high hardness, wear-resistance, and biocompatibility without the risk of bone growth on the articulating surfaces, ideal for joint