DUOC-01 protect against EAE. Average EAE scores of mice injected with Ringer's or DUOC-01 (N = 9 mice per group). Data are presented as mean $\pm$ SEM.

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A synthetic heparan sulphate mimetic for enhancing BMP-2-mediated osteogenesis and bone regeneration

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Background & Aim The bone morphogenetic proteins (BMPs) are a family of morphogens belonging to the TGF- β superfamily. BMP-2, a potent inducer of osteogenesis *in vitro* and *in vivo*, has been subject to extensive research for use in bone repair applications. Previously our lab has studied the effects of heparan sulphate (HS), a highly sulphated linear polysaccharides with a complex and heterogeneous structure, on the bioactivity of BMP-2 *in vitro* and *in vivo*. Whilst HS displays great potential for therapeutic applications in regenerative medicine and bone repair, animal-derived HS species are less desirable when compared to recombinant or synthetically-derived molecules. In the present study, a panel of 15 fully synthetic HS mimetics were screened against BMP-2 in an effort to identify potential targets for therapeutic applications in bone repair.

Methods, Results & Conclusion Of the 15 candidates, a four-armed dendrimer functionalized with oversulphated maltose residues and tetrahedral geometry (termed 'HS20') displayed the highest affinity toward BMP-2 via SPR completion and greatest enhancement BMP-2 bioactivity *in vitro*. Interestingly, the monomeric equivalent displayed little ability to bind, stabilize and enhance the bioactivity of BMP-2. Further analysis by fluorescence anisotropy and differential scanning fluorimetry confirmed HS20 is capable of avidly binding BMP-2. Moreover, HS20 was able to enhance association between BMP-2 and BMPRII in a dose-dependent manner. *In vitro* assays indicated that

HS20 is capable of enhancing BMP-2-mediated Smad 1/5/9 phosphorylation, osteogenic gene transcription and alkaline phosphatase activity in murine C2C12 cells. HS20 was also able to enhance calcium deposition in Human MSCs during osteogenic differentiation. Additionally, inhibition assays indicated that the pro-osteogenic effects of HS20 can be inhibited through small molecule-mediated BMPRII antagonism. Finally, HS20 was able to enhance *in vivo* bone formation in a critical-sized rabbit tibia defect over scaffold alone.

These data suggest that a fully synthetic short-armed tetramer bearing oversulphated maltose residues could function as a HS mimetic in bone regeneration applications. The synthesis of this compound is cost-effective and scalable, giving it great potential for translation of this fully synthetic HS mimetic into a clinical setting for bone regeneration applications.

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An allogenic 3D scaffold-free tissue engineered product for deep thickness skin regeneration: in vitro development to in vivo proof of concept

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Background & Aim Deep thickness skin wound remains a major challenge for reconstructive surgery. A novel approach of tissue engineering, based on an allogeneic adipose-derived 3D scaffold-free technology, was proposed.

Methods, Results & Conclusion **Material and Methods** Adipose-derived stromal cells (ASCs) were isolated from human adipose tissue to constitute the 3D-scaffold free graft by the production of the extracellular matrix (ECM, n=9). The ultrastructure of the graft was assessed by microtomography/SEM. The protein and growth factors contents were determined by proteomic analysis (LC-MS/MS) and ELISA, respectively. The *in vivo* biocompatibility (inflammatory reaction, biodegradation) was assessed in nude and Wistar rats up to 4 weeks (n=20) as well as the safety in terms of tumorigenicity/toxicity/biodistribution. The efficacy was then evaluated in a xenogenic (human to rat) model of ischemic (vs. non-ischemic) wound in hyperglycemic Wistar rats (n=42, 3D grafts vs. sham/Ctrl+).

Results The 3D-graft is a translucent and malleable membrane with a mean of 175 ± 86 cells/mm² found to be embed in the ECM with a low level of mineralization ($0.30 \pm 0.31\%$ v/v). The proteomic and genes analysis revealed the stimulation of the biological pathways involved in early wound healing and the over-expression of pro-angiogenic genes (ANG, ANGPT1, EPHB4, VEGFA, VEGFB, VEGFC, EDN1, THBS1, PTGS1, LEP) in the graft (in comparison to ASCs alone), respectively. The VEGF and SDF1a contents (181 ± 12 and 663 ± 27 ng/g, respectively) were also improved in the scaffold-free implant. The biocompatibility and the safety of the 3D-graft were confirmed at 4 and up to 24 weeks post-implantation, respectively. The 3D-graft was easily handled and applied by a simple bandage on the ischemic/hyperglycemic wounds (on the leg) and promoted an earlier irreversible wound closure (27 vs. 34 days for sham, respectively) associated with angiogenesis, dermis/epidermis reconstruction, transient and reversible increase of α SMA, lymphocytes/macrophages recruitment at 10-15 days.

Conclusion The scaffold-free approach with allogenic 3D-graft (derived from ASCs) demonstrated the safety and efficacy in stringent xenogenic model of hyperglycemic and ischemic deep-thickness wound.