Assessment of Novel Surgical Procedures to Regenerate Bone Using Decellularized Muscles& Bioactive Ceramic: A Histological Analysis

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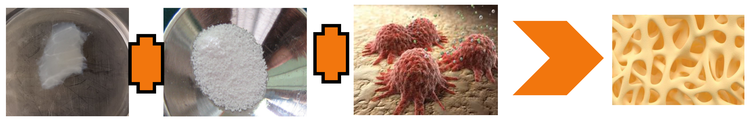
Introduction

Autogenous bone onlay, reported success rates range between 84% and 93.3% (Rana et al., 2011)(Chiapasco et al.2008).

However, the immediate bone resorption rate reached up to 50% due to lack of neovascularization in critical-sized bone defects (Szpindor, 1995)

Hypothesis

- Using bovine Decellularized muscle extracellular matrix (DSM) incorporating SCPC, then seeded with (hBMSCs) would augment calvarial bone in nude mice..



Materials & Methods&

CL1 -the immortalized human bone marrow stromal cells was used.

DSM preparation: Bovine semitendinosus and longissimus dorsi muscles (Porzionato et al., 2015) .

Bone bioactive Silica Calcium Phosphate Cement **SCPC** ceramic is used

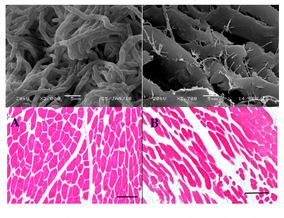
Materials and Methods *In vito Assessments*

Scanning electron microscopy **(SEM)**

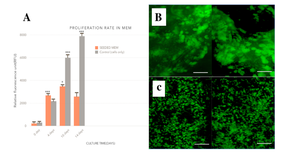
Viability test **(live and dead staining) and AlamarBlue®** proliferation assay

Histological assessment for DSM and the seeded DSM

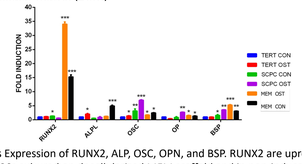
Quantitative reverse transcription-polymerase chain reaction **(qRT-PCR)**



Scanning electron microscopy and histology slides of decellularized muscle (DSM) tissues Showing decellurization of skeletal muscle .



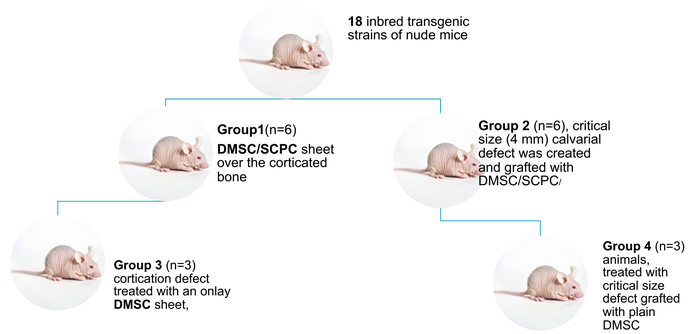
AlamarBlue proliferation assays of hBMSCs cultured on muscle extracellular matrix (DSM)



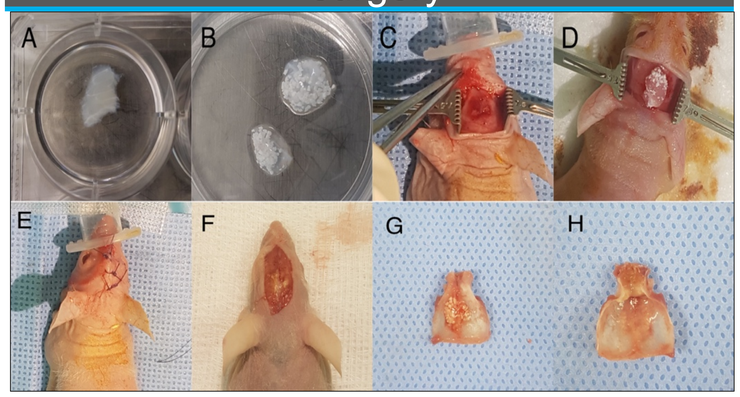
Molecular marker`s Expression of RUNX2, ALP, OSC, OPN, and BSP. RUNX2 are upregulated 35-fold and 15-fold in hBMSCs cultured on decellularized MEM scaffold and in non-induced hBMSCs

Materials and Methods *In vivo*

Two surgical defect`s models were created in 18 nude transgenic mice.



surgery



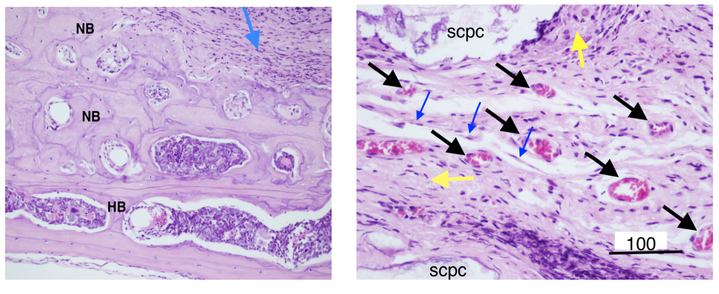
Data collection:

Histology study H&E, Masson`sTrichrome stains

Immunohistochemistery& Histomorphometry

Results

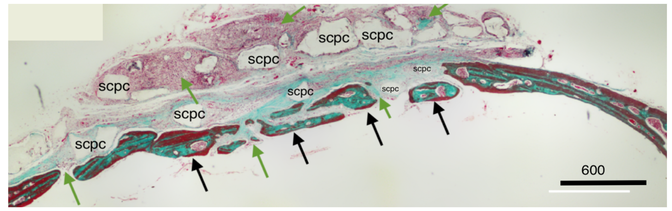
New bone formation and maturation was superior in groups treated with DMSC/SCPC/hMSC compared with those treated with DSM alone.



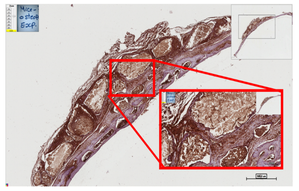
Photomicrograph showing the thickness of the host cranial bone (HB) next to the grafted issue(blue arrow) showed more New Bone(NB) regeneration reach more than double the thickness (of unoperated bone. Vascular and cellular noted at remnant of DSM.

Immunohistochemistry& Masson`s trichrome stains

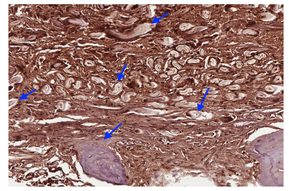
The newly formed bone in **group 1** and **group 3** was highly vascularized, showing mineralized collagen and high expression of ostepontine



Numerous blood vessels (black arrows) are recognized close to the SCPC particles. Islands of mature bone can be seen surrounded by high cellular activity and immature bone and loose connective tissue as periosteium like structure(blue arrow), Scale bar=100μm



Photomicrograph showing osteopontin staining for woven bone formation through the entire thickness of the onlay DSM/SCPC/hBMSC sheet graft. The onset demonstrates direct bone formation on the surface and in between the SCPC particles. The dark staining of the cells indicates differentiated osteoblasts

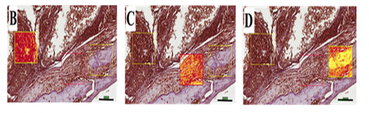


Higher magnification for the centre of the defect showing numerous bone spicules (blue arrows) surrounded with connective tissue that shows positive intake of osteopontin (OPN) immunostainin

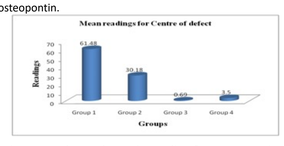
Histomorphometry

High expression of ostepontine was reported for group 1 &3

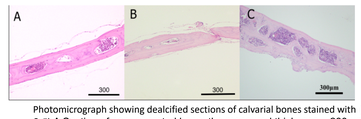
The newly formed bone was thicker and contained twice as much bone marrow spaces compared with control untreated bone



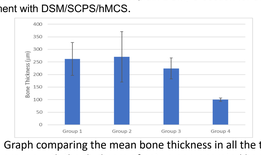
Photomicrograph showing colour deconvolution of selected areas in the cranial bone defect sections immune-stained with osteopontin.



Histogram shows the main reading for osteopontin stain at the center of the defects.



Photomicrograph showing dealcified sections of calvarial bones stained with (H & E),A Section of non- operated bone; the measured thickness = 280 μm. B Bone section after treatment with plain DSM; C section for defect after treatment with DSM/SCPS/hMCS.



Graph comparing the mean bone thickness in all the tested groups with the thickness of native non-operated bone (100 μm). A significant difference was found at p-value < 0.05

Conclusion

- New bone formation and maturation was superior in groups treated with DSM/SCPC/hMSC. The DMS/ SCPC scaffold has the ability to augment and induce bone regeneration and neovascularization in cases of major bone resorption and critical size defects.

Works Cited