Diabetic Complications Consortium

Application Title: A Robust Microphysiological System for Mechanistic Investigation of Fat-bone Crosstalk in Diabetes.

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1. Project Accomplishments:

1. Generate the bioreactor to host the bone and fat tissues
2. Successfully generate bone tissues, which contains both osteoblasts and osteoclasts
3. Successfully generate adipose tissues using stem cells from healthy donors
4. Collect the stem cells from diabetic patients
5. Create fat pad from diabetic stem cells

2. Specific Aims:

Specific Aim 1. Define the impact of fat on bone health using NORMAL μAD/μBO complex tissue chips.

Results

To generate normal bone and fat tissues, adipose-derived stem cells (ASCs) were encapsulated within the GelMA scaffolds and subjected to the differentiation culture. To elucidate the optimal osteogenesis time point, we examined the expression levels of osteogenic markers, as well as performed the histology staining after 14, 21, 28 days differentiation. As shown in **Figure1A**, quantitative RT-PCR assay showed osteogenic markers (RUNX2, ALP, OCN, OSX, BSP2) were all upregulated compared to those in control medium and the peak of mRNA expression levels were at day 21. Furthermore, histological staining also confirmed that there were more calcium deposition than those cultured in control group and calcium deposition were highest on day 28 (**Figure1B**).

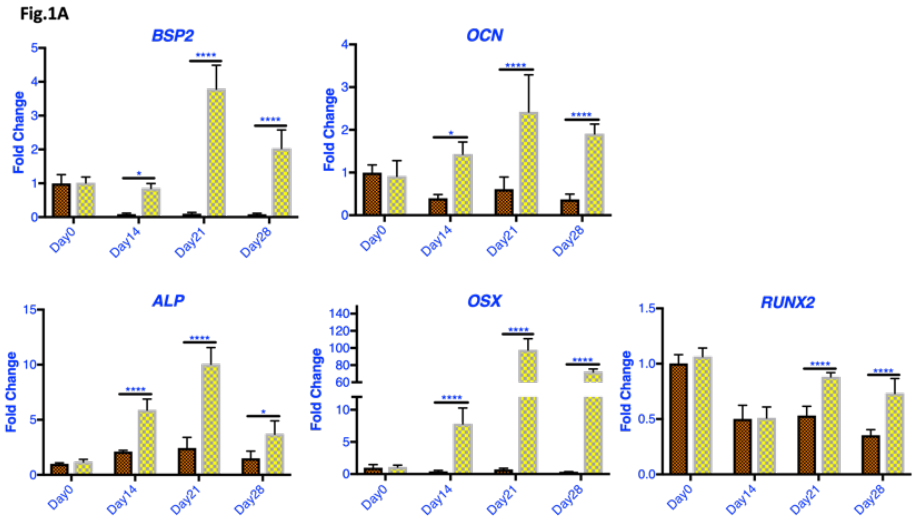

Fig.1A: Genes expression of ASCs-laden gelatin hydrogels cultured under osteogenic medium for 14, 21,28 days in comparison with those cultured under growth medium. N=4-5, Orange bar: osteogenic medium group; yellow bar: growth medium group. * P<0.05, ** P<0.01, *** P<0.001, ****P<0.0001

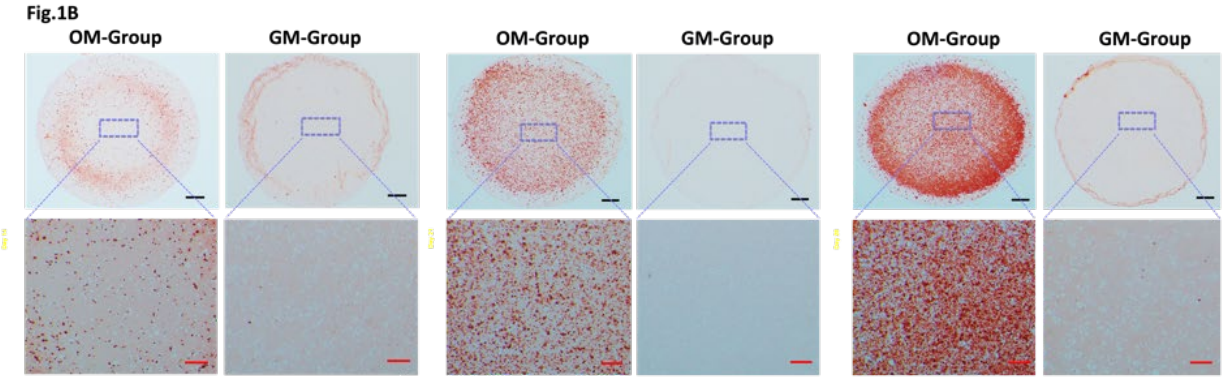

Fig.1-B: Alizarin red staining of ASCs-laden gelatin hydrogels cultured in osteogenic medium or growth medium for 14, 21, 28 days. GM: growth medium, OM: osteogenic medium; black scale bar: 500μm; red scale bar: 200μm

Taken together, our results indicate that human ASCs could be successfully differentiated toward osteogenic lineages with appropriate stimulation and the optimal differentiation time is 21-day.

Similar to osteogenesis, quantitative RT-PCR assay showed adipogenic markers (PPARγ1, PPARγ2, LPL, adipsin and adiponectin) were all upregulated compared to those in control medium and the peak of mRNA expression levels were appeared at 21 days differentiation (**Figure2A**). In particular, the expression level of adiponectin reached the plateau at day 14 and maintain up to 28 days. Histology staining also confirmed that there were significantly more lipid deposition than those cultured in control group and lipid deposition were highest on day 28 (**Figure2B**). Taken together, our results indicated that human ASCs have been successfully differentiated toward adipogenic lineages with appropriate stimulation. The optimal differentiation time is 21 day.

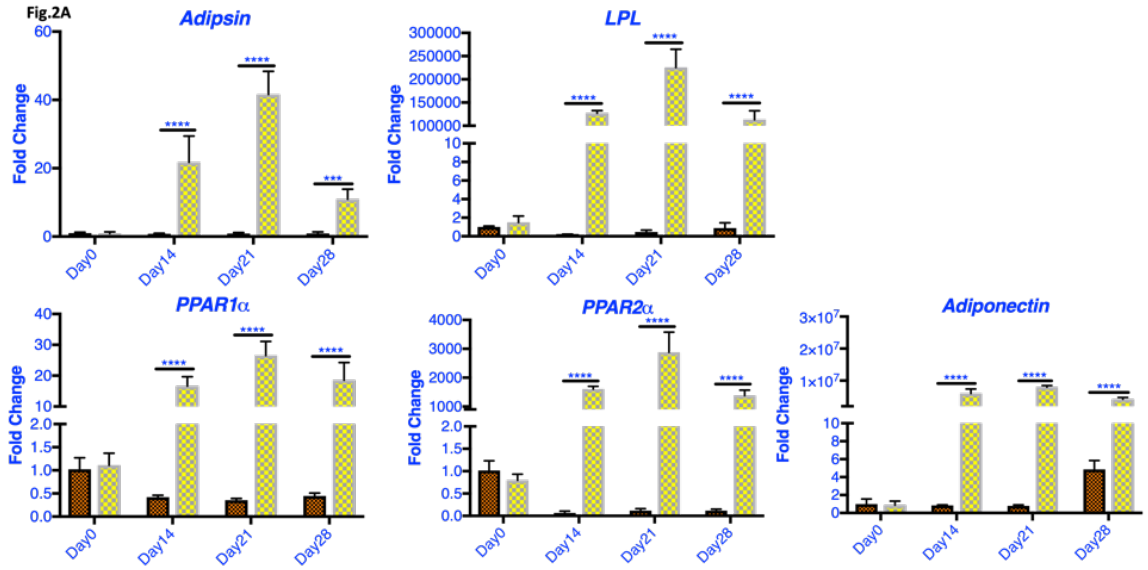

Fig.2A: Genes expression of ASCs-laden gelatin hydrogels cultured under adipogenic medium for 14, 21,28 days in comparison with those cultured under growth medium. N=5-6, Orange bar: adipogenic medium group; yellow bar: growth medium group. * P<0.05, ** P<0.01, *** P<0.001, ****P<0.0001

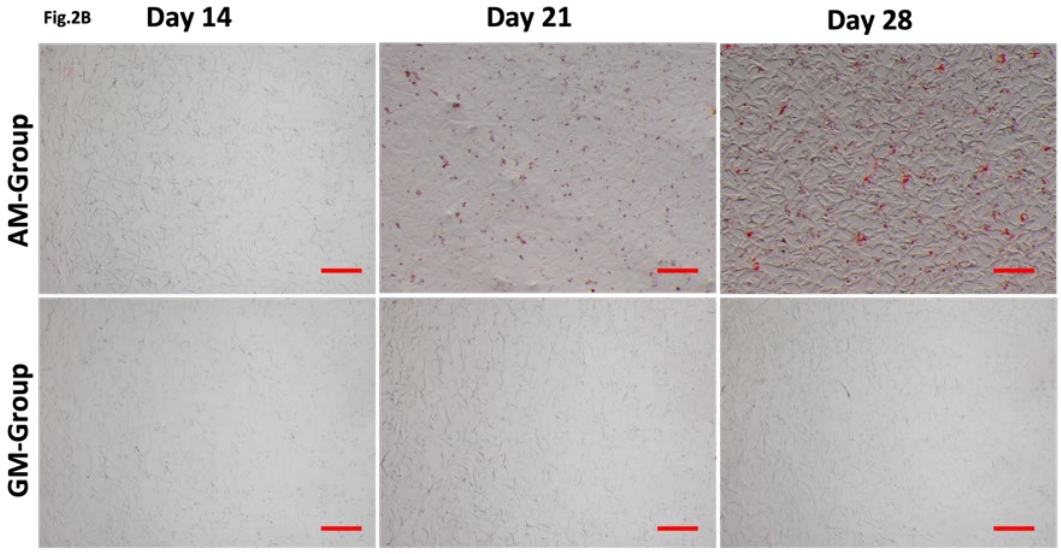

Fig.2-B: Oil red staining of ASCs-laden gelatin hydrogels cultured in adipogenic medium or growth medium for 14, 21, 28 days. GM: growth medium, AM: adipogenic medium; red scale bar: 200μm

The osteoclastogenesis of monocytes had also been assessed with real time PCR. As shown in **Figure 3**, robust increase of osteoclast marker genes have been elevated after induction. Currently, we are working on histology to further confirm the 3D osteoclastogenesis within the scaffolds.

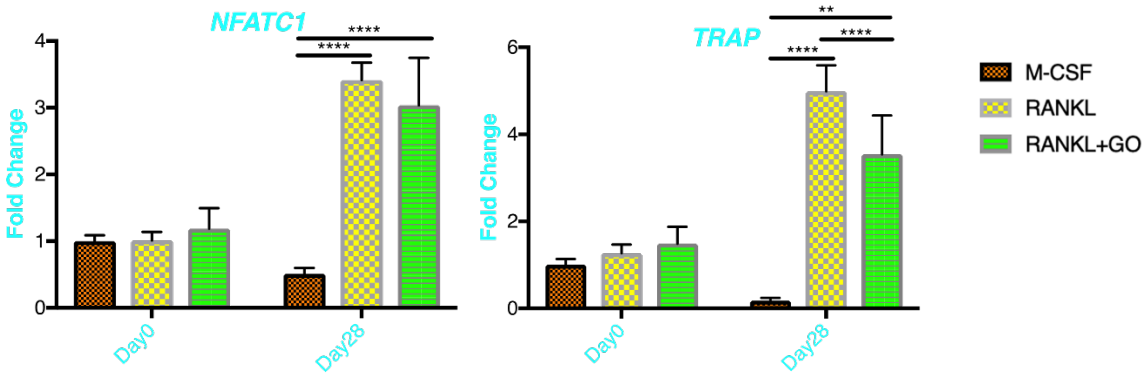

Figure 3. Real time PCR after 28 days osteoclastogenesis with different inducing methods. The application of RANKL-containing medium is the best osteoclastogenic medium.

Specific Aim 2. Test potential adipose-targeting therapeutics on enhancing the health of T2D adipose and bone.

Results: ASCs from non-diabetic, pre-diabetic, and diabetic IPFP, or healthy subcutaneous fat (Subcue) were isolated and expanded. To generate adipose tissues, these cells were seeded into GelMA scaffolds at 20M cells/mL. Scaffolds were cultured for 56 days in growth or adipogenic media. Live/Dead staining demonstrated high levels of cell viability. Oil Red O staining demonstrated accumulation of lipid droplets within the scaffolds cultured within adipogenic media (**Figure 4**) and RT-qPCR analysis of mRNA demonstrated upregulation of the expression of genes indicating terminal differentiation into adipocytes (**Figure 5**). Interestingly, cells from prediabetic or diabetic donors has generated significantly less adiponectin than healthy control.

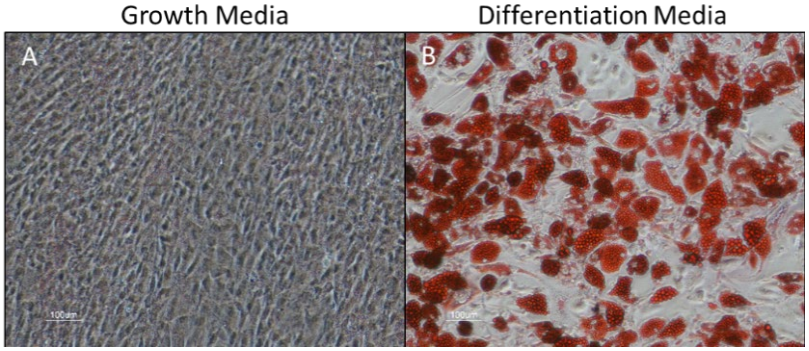

Figure 4. Oil red staining

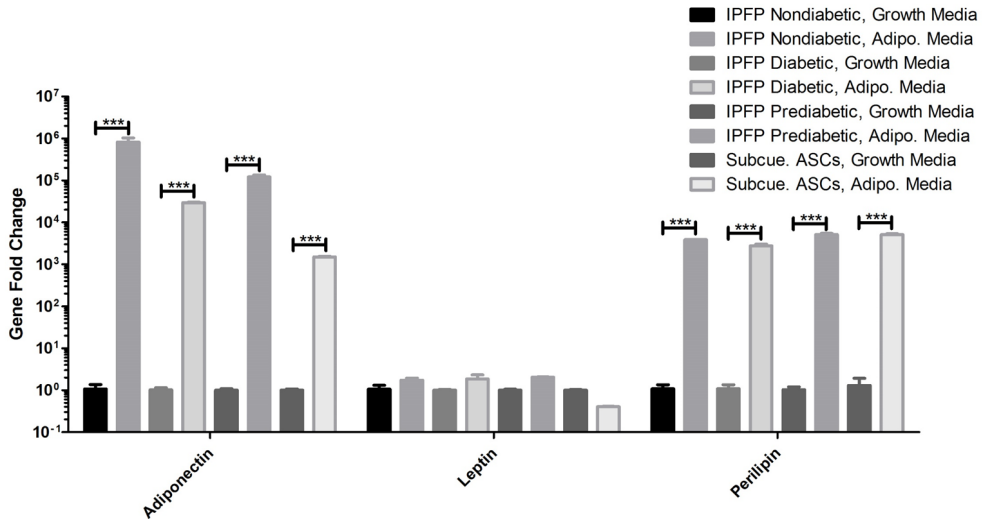

Figure 5. Real time PCR for the adipose tissues generated from ASC isolated from nondiabetic, diabetic and prediabetes donors.

3. Publications:

None.