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Bone marrow adipocytes in 3D

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Standfirst

Culturing bone marrow stromal cells on 3D silk scaffolds supports their proliferation and adipogenesis, while minimizing the activation of inflammatory pathways. Therefore, differentiation of bone marrow adipocytes in 3D culture might provide a more representative model for the study of bone marrow adipose tissue than is offered by traditional 2D cell cultures.

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Adipose tissue is an important endocrine organ that secretes hormones and cytokines, which regulate many physiological processes, including food intake, energy expenditure, insulin sensitivity and inflammation. However, not all adipose depots are created equal — the morphology and physiology of adipocytes varies substantially depending on the location of the adipose depot in which they reside. White adipose tissues are the most abundant adipose depots in the body and serve as the primary site of energy storage. Although brown and beige adipose tissues are less common than white adipose tissues, they have received considerable attention because of their potential to consume energy through non-shivering thermogenesis, which is an important potential target for obesity therapy. The adipose depot within the bone marrow, termed bone marrow adipose tissue, is unique because its volume is constrained and it has local interactions with multiple cell types, including haematopoietic, osteogenic, vascular and mesenchymal cells. Bone marrow adipose tissue is also the source of a disproportionate fraction of circulating adiponectin and, through its endocrine effects, is required for the metabolic adaptation of skeletal muscle to caloric restriction^{1,2}. The study of

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Competing interests statement

The authors declare no competing interests.

bone marrow adipose tissue *in vivo* is complicated by its inaccessibility (that is, its encasement within bone), which perhaps explains why this depot is the subject of fewer than 0.01% of publications in the field of adipose research, despite bone marrow adipose tissue comprising approximately 10% of adipose mass in healthy adults¹.

The development of an *in vitro* system that accurately and reproducibly models the cellular physiology of bone marrow adipose tissue *in vivo* would greatly enhance our ability to explore the unique molecular and metabolic characteristics of bone marrow adipocytes and their functional interactions with other cell types. The use of 2D cultures of immortalized adipocytes or of primary adipocytes has provided fundamental insights into the mechanisms of differentiation and metabolism in adipose tissue, insights that have by and large held true for adipocytes within adipose tissues *in vivo*. In addition, 2D co-culture of primary adipocytes with other cell types, including macrophages and tumour cells, has helped to define the complex paracrine interactions that occur in adipose tissue³. However, 2D cell culture has several drawbacks, including forced cell polarity, increased cell stiffness, the lack of an enveloping extracellular matrix and an uneven distribution of focal adhesions in cells⁴. Therefore, for some applications, a 2D cellular environment hampers which experimental questions can be asked. To overcome the deficiencies of 2D cell culture, cells have been grown in 3D cultures, either in gel matrices or on filamentous scaffolds. In general, compared with cells cultured in 2D, cells cultured in 3D demonstrate increased survival, proliferation, cell type-specific gene expression and drug susceptibility⁵. Most importantly, the morphological and biochemical properties of 3D-cultured cells more closely resemble those of cells in their native tissues, and adipocytes are no exception⁵. Culturing immortalized mesenchymal stem cells in adipogenic cell culture media is a common method to differentiate adipocytes *in vitro* and when carried out in 3D on porous nanocellulose scaffolds, the cells show increased proliferation and differentiation into adipocytes⁶. In 3D cultures of mouse preadipocytes isolated from visceral white adipose tissue, a higher proportion of cells undergo adipogenesis, and the resulting adipocytes more faithfully retain depot-specific molecular characteristics than adipocytes cultured in 2D⁷.

The study by Fairfield and colleagues builds on previous developments in tissue-engineered cell culture to establish a 3D culture model of bone marrow adipocytes⁸. Bone marrow cells derived from mice or humans were cultured in 3D on porous silk fibroin scaffolds, a setup that supported cell proliferation and differentiation into lipid-containing adipocytes. Furthermore, the cell cultures remained stable for at least three months, as demonstrated by the presence of live adipocytes with unilocular lipid droplets⁸. Thus, the possibility now exists for long-term *in vitro* studies of bone marrow adipocytes and stromal cells.

The researchers further characterized the marrow-derived stromal cells and adipocytes in their 3D culture system. Microarray analysis of differentiated bone marrow stem cells demonstrated that cells grown in this 3D culture had striking differences in gene expression compared with cells cultured in 2D⁸. Pathway analysis of microarray data using the Kyoto Encyclopedia of Genes and Genomes (KEGG) showed that genes involved in metabolic and proliferation pathways were upregulated in cells in 3D culture, whereas genes involved in inflammation and disease-related pathways were downregulated. These results agree well with previous observations that 3D cell culture favours the proliferation, differentiation and

prolonged survival of cells^{5,9}. It is well established that chronic low-grade inflammation, such as that present in obesity, leads to adipocyte and metabolic dysfunction. Therefore, selecting a model for adipocyte cell culture that minimizes activation of inflammatory pathways might be advantageous. The alterations in gene expression observed in this study were mirrored by significant differences in protein expression between 2D-cultured and 3D-cultured bone marrow stem cells⁸. Interestingly, proteomic analyses revealed that the protein expression pattern of cultured bone marrow cells, regardless of whether they were differentiated in 2D or 3D culture, diverged substantially from that of native adipose tissues. Whereas these differences might exist simply because *in vitro* culturing of cells alters their expression of proteins, the differences might also be due to using a mixed culture of cells that lacks the full complement of cell types present within the tissue, or due to comparing bone marrow cultures to white and brown adipose tissues rather than to bone marrow adipose tissue⁸.

The researchers also showed that, in co-cultures of myeloma cells with scaffold-supported bone marrow adipocytes, the cancer cells accumulated lipids⁸. This result, together with the observation that co-culture with myeloma cells resulted in delipidation of bone marrow adipocytes, suggests that lipids might be transferred from adipocytes to growing myeloma cells. Understanding the metabolic interactions between bone marrow adipocytes and myeloid tumour cells could provide novel targets to interrupt the metabolism and thereby limit the growth of myeloma cells.

The development of this 3D cell culture model of bone marrow adipose tissue is clearly a step forward for research efforts to understand marrow adipocyte biology. Looking ahead, the challenge will be to establish an accurate model of bone marrow adipose tissue that includes skeletal cells, haematopoietic cells and other cell types present in the bone marrow. However, this task might be more complex than anticipated. A recent study¹⁰ using 3D electron microscopy reported that perivascular endothelial cells extend complex dendrite-like projections into the surface of bone marrow adipocytes, and that each bone marrow adipocyte can contact >100 haematopoietic cells, which might utilize free fatty acids from the adipocytes, similar to co-cultured myeloma cells⁸. In the interim, 3D culture models seem to provide considerable advantages over traditional 2D cell culture methods, and should improve the quality of mechanistic investigations of the biology of bone marrow adipose tissue.

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References

1. Scheller EL, Burr AA, MacDougald OA & Cawthorn WP Inside out: bone marrow adipose tissue as a source of circulating adiponectin. *Adipocyte* 5, 251–269 (2016). [PubMed: 27617171]
2. Cawthorn WP et al. Bone marrow adipose tissue is an endocrine organ that contributes to increased circulating adiponectin during caloric restriction. *Cell Metab.* 20, 368–375 (2014). [PubMed: 24998914]

3. Santander AM et al. Paracrine Interactions between adipocytes and tumor cells recruit and modify macrophages to the mammary tumor microenvironment: the role of obesity and inflammation in breast adipose tissue. *Cancers (Basel)* 7, 143–178 (2015). [PubMed: 25599228]
4. Baker BM & Chen CS Deconstructing the third dimension: how 3D culture microenvironments alter cellular cues. *J. Cell Sci* 125, 3015–3024 (2012). [PubMed: 22797912]
5. Ravi M, Paramesh V, Kaviya SR, Anuradha E & Solomon FD 3D cell culture systems: advantages and applications. *J. Cell. Physiol* 230, 16–26 (2015). [PubMed: 24912145]
6. Krontiras P, Gatenholm P & Hagg DA Adipogenic differentiation of stem cells in three-dimensional porous bacterial nanocellulose scaffolds. *J. Biomed. Mater. Res. B Appl. Biomater* 103, 195–203 (2015). [PubMed: 24819827]
7. Emont MP et al. Using a 3D culture system to differentiate visceral adipocytes in vitro. *Endocrinology* 156, 4761–4768 (2015). [PubMed: 26425808]
8. Fairfield H et al. Development of a 3D bone marrow adipose tissue model. *Bone* 10.1016/j.bone.2018.01.023 (2018).
9. Alessandri M et al. Influence of biological matrix and artificial electrospun scaffolds on proliferation, differentiation and trophic factor synthesis of rat embryonic stem cells. *Matrix Biol.* 33, 68–76 (2014). [PubMed: 23954537]
10. Robles H et al. Characterization of the bone marrow adipocyte niche with three-dimensional electron microscopy. *Bone* 10.1016/j.bone.2018.01.020 (2018).