Cultured Meat with Tunable Fat Content: Scaffolds for Multicomponent Tissue with Myocytes and Adipocytes

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Statement of Purpose: Alternative methods for meat production are needed to increase the sustainability and resiliency of our food system[1]. Cultured meat has potential to reduce dependence on livestock agriculture, however, innovations are needed to increase the palatability and scalability of cultured meat[2]. Interspersed fat within muscle tissue, or fat marbling, is an essential contributor to meat mouthfeel and flavor; however, recapitulating fat marbling in vitro is challenging and requires co-culture of both myocyte and adipocyte cells on scaffolds that support the growth and maturation of both cell types. Engineering tunable scaffolds provides a strategy to increase the culturing efficiency of myocytes and adipocytes, which are both sensitive to mechanical cues[3,4]. To increase culture efficiency of myocytes, we have developed scaffolds that have physiologically-relevant stiffness and striated topographies of natural muscle that drive the differentiation of myocytes into myotubes. We also tuned our edible scaffolds to support the growth of adipocytes in suspension culture. To further enhance the scalability of cultured meat, we additionally developed edible microcarrier scaffolds with grooved and aligned topology to increase the efficiency of culturing and differentiating muscle cells in a single bioreactor system. Here we present strategies to develop scaffolds with tunable physical properties that support the production of cultured meat with tunable fat content.

Methods: To engineer structured cuts of cultured meat with tunable fat content, we generated aligned nanofibers via electrospinning gelatin onto a rotating cylindrical collection device. To generate edible microcarriers with defined stiffness to support both myocytes and adipocytes, we used water-in-oil emulsions as a scalable approach to fabricate gelatin beads crosslinked with transglutaminase. Myoblasts were proliferated and differentiated on aligned nanofibers, while adipocytes were grown and differentiated on spherical gelatin microcarriers. Post differentiation, muscle and fat microtissues were assembled and adhered to one another to form marbled cultured meat with tunable fat content. To fabricate microcarriers with striated surface texture, we also developed a novel embossing technique to imprint edible microcarriers with a smooth surface (sMCs) with a striated topology to form grooved microcarriers (gMCs). Muscle cells were proliferated and differentiated on both sMCs and gMCs, and compared to commercially available Cytodex beads (Fig 1b). The resulting muscle microtissues were then centrifuged and further crosslinked with transglutaminase, resulting in homogenous cultured meat.

Results: Our approach to engineer cultured meat supports the development of structured tissue with differentiated muscle and fat, which forms a mechanically stable structure without the use of additional crosslinkers (data not shown). In addition, we were able to engineer thicker ~2 mm tissue with both muscle on aligned nanofibers and fat on edible microcarriers; the components adhered into a cohesive structure on the timescale of hours, thus circumventing diffusion limitations. These findings demonstrate the feasibility of using a bottom-up approach towards engineering thick, structured cuts of marbled cultured meat with tunable fat content. Edible microcarriers with both smooth and grooved surface topologies supported the proliferation and differentiation of mouse myogenic C2C12 cells and the growth of primary bovine satellite muscle cells in a suspension culture.

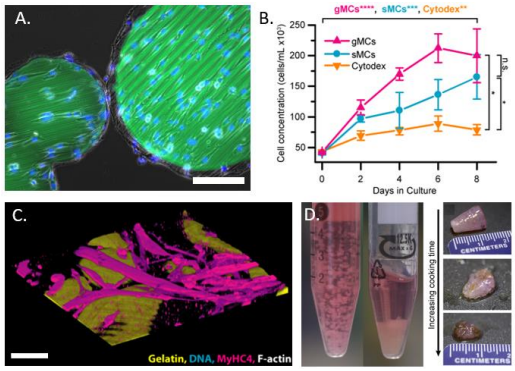

Figure 1: (a) C2C12 cells aligning on edible grooved microcarriers (green) stained with Hoechst (blue); Scale bar: 200 µm. (b) Proliferation of C2C12 on grooved microcarriers (gMCs) compared to C2C12 grown on spherical microcarriers (sMCs) and Cytodex microcarriers. (c) Confocal imaging of C2C12 myotubes on gMCs (yellow) and stained with Myosin Heavy Chain (magenta); Scale bar: 200 µm. (d) Proof-of-concept cooking demonstration of bovine satellite muscle cells grown on sMCs and harvested via centrifugation.

To demonstrate feasibility of edible microcarriers for cultured meat, we showed that edible scaffolds can support the production of myogenic microtissue from C2C12 or bovine satellite muscle cells, which we harvested by centrifugation. The resultant cultured beef 'patties' maintained their shape during cooking and exhibited browning characteristic of Maillard reactions (Figure 1d). These findings demonstrate the potential of edible scaffolds for the scalable production of cultured meat.

References: [1] Nijdam, D., et al. Food Policy. 2012;37:760–770.; [2]Tomiyama, A.J., et al. Trends in Food Science & Technology. 2020; 104:144-152. [3]Romanazzo, S., et al. Sci. technol. Adv. Mater. 2012;13:1-9; [4]Young, D.A., et al. Biomaterials. 2013;34:8581-8588.