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Review Article

The important role of cellular mechanical microenvironment in engineering structured cultivated meat: Recent advances

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ABSTRACT

Cultivated meat (CM) provides a potential solution to meet the rising demand for eco-friendly meat supply systems. Recent efforts focus on producing CM that replicates the architecture and textural toughness of natural skeletal muscle. Significance of the regulated role of cellular microenvironment in myogenesis has been reinforced by the substantial influence of mechanical cues in mediating the muscle tissue organization. However, the formation of structured CM has not been adequately described in context of the mechanical microenvironment. In this review, we provide an updated understanding of the myogenesis process within mechanically dynamic three-dimensional microenvironments, discuss the effects of environmental mechanical factors on muscle tissue regeneration and how cell mechanics respond to the mechanical condition, and further highlight the role of mechanical cues as important references in constructing a sustainable Hydrocolloids-based biomaterials for CM engineering. These findings help to overcome current limitations in improving the textural properties of CM.

1. Introduction

Livestock grazing has rapidly expanded with the increasing global trends in meat consumption. However, this conventional meat production system has a link to mass animal slaughter, resource-intensive technologies, and negative public health impacts have arisen due to the prospect of zoonotic pathogens (Bomkamp and Skaalure, 2022). In its stead, several other sustainable techniques are under development that are expected to promote the sustainability of the animal-sourced protein supply systems.

Tissue engineering allows for the specific recapitulation of natural processes involved in the regeneration of skeletal muscle and mesenchymal tissues. In 2002, National Aeronautics and Space Administration (NASA) produced the first lab-grown fish muscle with the aim of providing viable means for supplying safe, healthy, and nutritious space food (Benjaminson et al., 2002). Cultivated meat (CM), generated from animal cells instead of the entire animal, has since emerged as an alternative to conventional meat supply. The lab-grown approach has the potential to address concerns about meat equivalents; to reduce environmental, ethical, and public health problems associated with large-scale animal agriculture (Yap et al., 2023). Furthermore, CM lends to engineering procedures that have the potential to boast healthier and more specialized meat options.

Newly produced CM is expected to meet organoleptic standards comparable to conventional meat. This highlights the need to consider the native architecture, texture, and toughness of muscle tissue. Understanding the maturation of muscle fiber, fat, and the spatiotemporal organization of connective tissue from the perspective of tissue engineering can offer a substantial contribution to improving the quality of CM products (Bomkamp and Skaalure, 2022; Seah et al., 2022a). One of the main mechanical determinants of connective tissue is extracellular matrix (ECM), which creates a critical microenvironment that promotes and regulates myogenic cell adhesion, growth, and survivability. The increasing knowledge base has led to deciphering regulatory parameters that are inherent of the microenvironment and its biomechanics. The resident myogenic cells can sense these distinct microscopic organizational biomechanics of the ECM, leading to the development of complex organized ECM-cell structures that grant the mechanical complexity to

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muscles. Utilizing the effect of the mechanical microenvironment helps to reach the necessary attributes in the final product of CM, notably the texture. However, the advancement of the key roles of cellular mechanical microenvironment on myogenesis has not been comprehensively discussed in context of the generation and textural improvement of CM. Therefore, in this review, we emphasize the imperative interplay between the mechanical microenvironment and the formation of CM, with the intention to inspire prospective solutions to the existing constraints of remodeling the natural structure and organoleptic aspects when engineering CM.

2. Basic technique requirements

Efficient production of CM depends on three main technological aspects: (1) the isolation and initial cultivation of cell sources from animals, (2) myofiber maturation on appropriate scaffolds, and (3) processing into food products (Ng and Kurisawa, 2021). Significant advances have been garnered in muscle tissue engineering. However, technical specifications for initial cell cultivation and 3D tissue organization should be tailored to the food bioprocessing industry, while ensuring that the product has a biomimetic texture resembling that of traditional meat and is safe for human consumption (Levi et al., 2022).

2.1. Cell sources

Prominent CM-generating seed cells are those that possess self-renewal, proliferation, and differentiation potential, such as: (a) muscle precursor cells including myoblasts and satellite cells; (b) adipose-derived stem cells (ADSCs); (c) mesenchymal stem cells (MSCs); (d) embryonic stem cells (ESCs); and (e) induced pluripotent stem cells (iPSCs) (Singh et al., 2023a). Satellite cells (SCs) are frequently employed for the development of mature myofibrils during meat generation (Bomkamp and Skaalure, 2022). ADSCs, which are optional "fat-seeds", have the tendency to develop into pre-adipocytes, which in-turn eventually differentiate into mature adipocytes. Priority should be given to the working scale, number, stemness, and viability of the seed cell population (Guan et al., 2022). Numerous new insights into cellular heterogeneity, with respect to stem cell pluripotency, self-renewal capacity, and other traits, have been presented recently. Current approaches for cell sorting and quality control that primarily focus on phenotypic parameters may be limiting factors when seeking to exploit the resource value of seed cells (Giordani et al., 2019; Dell'Orso et al., 2019).

2.2. Animal-free media

The Development of animal-free media with efficacy and performance similar to serum-based media remains a challenging task. There is a lack of systematic studies on the combination of specific cytokines and nutrients tailored to meet the metabolic, survival, and adhesion needs of varying cell types (Reiss et al., 2021; Gomez Romero and Boyle, 2023). Commercial Xeno-free media, including FBM™, TesR™, and Essential 8™, have the potential to facilitate primary bovine myoblast growth. LipoGro™, which was originally developed for clinical-grade human stem cells, can also serve as a serum replacement. However, it has been proven inefficient at promoting cell growth and maintaining the myoblast phenotype at a level that is comparable to serum (Lindroos et al., 2009; Kolkmann and Post, 2020). In distinct serum-free media, such as B27 and AIM-V, proliferative myoblast cells exhibited alterations in the metabolic properties of myofibers, resulting in deviation to slow, intermediate, and fast phenotypes. Compared to the fast glycolytic type fiber markers that emerge in serum-based culture, C2C12 cells grown in AIM-V exhibited oxidative and glycolytic types, which are recognized as intermediate muscle types. The dominant type of cells grown in B27 media exhibited slow and oxidative metabolic phenotypes with high levels of non-essential amino acids and total amino acids. This indicated that serum substitutes serve as another crucial consideration for catering the growth and resulting taste of CM(Jang et al., 2022).

2.3. Nutritional and physical sensations

Ongoing fundamental research aims to optimize the nutritional and sensorial (texture, taste, and visual) aspects of CM. Conventional meat contains adequate amounts of essential amino acids, and is high in saturated fatty acids, minerals, and vitamins. Lipid-soluble compounds and water-holding capacity (WHC) give meat its distinct taste properties and juiciness (Broucke et al., 2023). Culture medium nutrients can trigger the synthesis of most cytoskeletal proteins, and certain fatty acids (and vitamins). However, despite aiding in the modification of the final product's nutritional profile, the implications of these remedies on human health require full understanding. The physicochemical properties of scaffolds and their processed materials are crucial in the synthesis of in vitro meat. The stability of biopolymers and synthesis methods are linked to the cytocompatibility, structural integrity, and shelf life of the CM scaffold. Specifically, surface morphology and stiffness play significant roles in regulating muscle cell adherence and proliferation (Singh et al., 2023b). The scaffolds also furnish a critical mechanical matrix for the differentiation and assembly of myofibrils, thereby directly contributing to the textural aspects of CM, such as tenderness and structure (Broucke et al., 2023). An absence of sufficient 3D mechanical support results in poorly organized tissue due to a reduced ability to control cell distribution (Thorrez et al., 2018). Therefore, comprehending the complex mechanism by which muscle cells interact with their mechanical microenvironment is very critical to rebuild the integrity of meat texture, as well as considerations for bioengineered scaffolds.

3. The mechanical microenvironment for myogenesis

The physiological process of myogenesis is constantly regulated by the tissue microenvironment, wherein various mechanical stress forces are exerted and are orchestrated to modulate fate of residing cells. This section places special emphasis on the mechanical microenvironment as an emerging concept for recapitulating the mechanical complexity of the ECM for myogenesis in the engineering of CM.

3.1. Hierarchical structure and biomechanics of skeletal muscle

The mechanical and textural performance of meat is tightly linked to the variations in the composition and structure of naturally occurring muscle tissue. This relationship has been elucidated in terms of anatomic structure, collagen sedimentation, and biomechanical strength. Engineering CM generally refers to the tissue engineering of skeletal muscle from various livestock and fish species. Skeletal muscle is approximately 90% muscle fibers, 10% connective tissue and adipose tissue, and less than 1% blood vessel tissue (Listrat et al., 2016a). The connective layers are characterized by the complex networks of ECM surrounding the muscle fibers. ECM networks consist mainly of fibrous proteins surrounded by proteoglycans (PGs) (Gillies and Lieber, 2011). Multinucleated myofibers represent the fundamental structural and functional unit within the skeletal muscle, with a range in length from 1 to 100 mm and a diameter from 10 to 100 μm (Lee et al., 2021). The myofibrils bundles constitute the majority of the intracellular volume of a muscle fiber, with the sarcomere arranged hierarchically in parallel to the longitudinal axis of the fiber (Fig. 1A). The elongated fibrillar structures of myofibers are mainly attributed to the abundance of myofibrillar proteins. These proteins serve not only as molecular motors for muscle contraction, but also impart distinctive mouthfeel to meat, due to their capacity to retain water, form gels and emulsify (Yan et al., 2022). Therefore, the characteristics of the contractile and metabolic types, size, number, density, and orientation of myofibers have a predominant impact on the fibrous texture and appearance of meat (Fish et al., 2020).

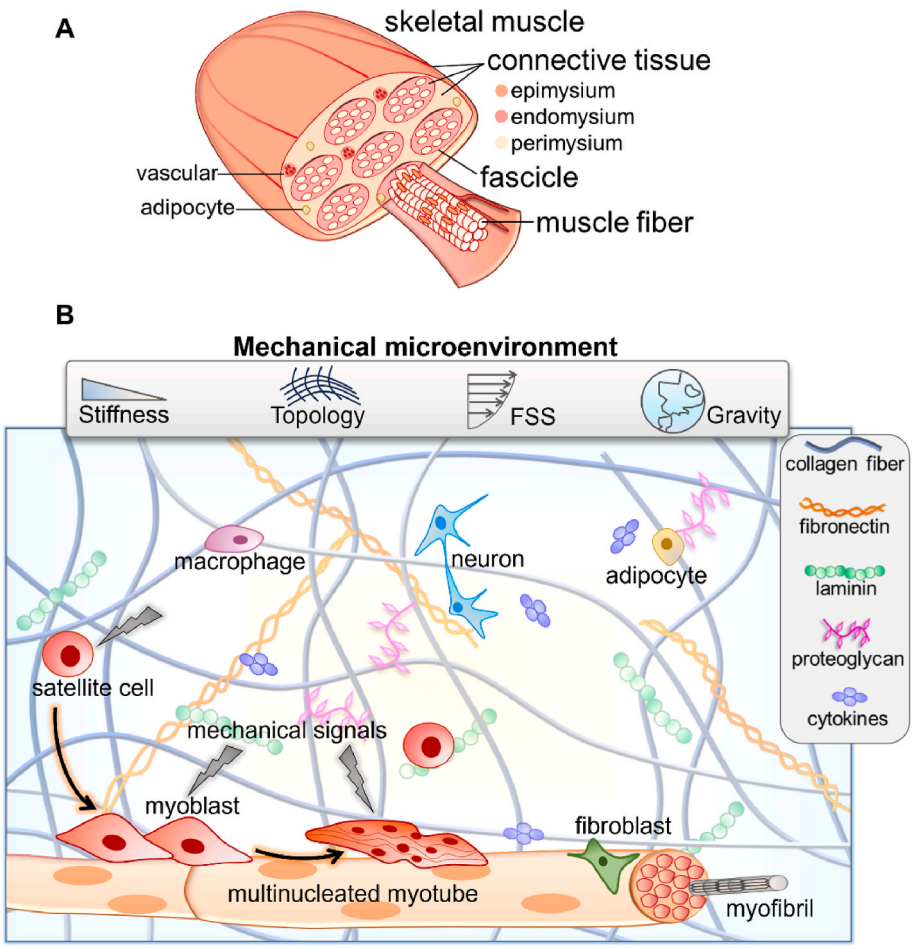

Fig. 1. Hierarchical structural model of the mammalian skeletal muscle systems at the multiscale level. A. Cross-section illustration highlights the main components of muscle tissue. Bundles of muscle fibers, individual myofibers and intramuscular fat are compartmentalized by connective tissues that collectively exist as epimysium (surrounding the muscle), perimysium (surrounding muscle fascicles), and endomysium (surrounding muscle fibers), respectively. B. The mechanical microenvironment of myogenesis. The ECM networks predominantly consist of collagens, which are the most prevalent ECM protein in mammalian connective tissues. The ECM backbone is the crucial microenvironment where myogenic cells reside and integrate various stimuli to determine their fate.

Environmental factors and molecular genetics can independently affect the biochemical and structural characteristics of these muscle components, allowing for the efficient modulation of meat quality (Listrat et al., 2016b). The biochemical composition and rearrangement of the ECM matrix that cross-linked by stable interchain bonds, are highly correlated with the stiffness, tenderness and toughness of meat. For example, the degree of collagen cross-linking is a key contributor for ECM stiffness and microstructure. The degree of intermolecular connectivity also enhances the structural density of the endomysium and perimysium, which determines the shear force and tensile strength of the raw meat (Nishimura, 2015; Lepetit, 2008).

3.2. The mechanical microenvironment of skeletal muscle

The ECM provides multifaceted microenvironments or niches that support development of myogenic cells. Tissue-specific cell populations, ECM components, varied cytokines are integrated together to control the process of myogenesis. The ECM serves as feedback systems for communicating information about the state of a tissue back to the SCs or myoblasts when tissue damage occurs (Ahmad and Lim, 2021; Jones et al., 2023). During myogenesis, these cells either fuse together or adhere to damaged fibers. The effective coordination of myoblast proliferation, migration, polarization, differentiation, and fusion across multiple length scales is necessary for the muscle regeneration. The regulation of such highly orchestrated cellular communication is tightly regulated through biochemical, structural, and mechanical cues that originate from the surrounding microenvironment. Biochemical cues (e.g. cytokines, extracellular vesicles, ECM proteins and other diffusible molecules) stimulate paracrine or autocrine signals that facilitate the muscle fiber growth (Dumont et al., 2015). Nevertheless, the formation of ordered myotube segments is determined by the intrinsic biophysical properties of the ECM and the transmission of extrinsic mechanical stimuli through it (Distler et al., 2020). Cell-derived ECM proteins and proteinase enzymes exist in equilibrium or imbalance that dictates the continuous ECM remodeling and maintenance of the homeostasis of myogenic programs (Fig. 1B) (Forcina et al., 2019; Loreti and Sacco, 2022).

3.2.1. The topographical microenvironment

The morphological architecture of natural muscle tissue comprises well-organized myofibers which are surrounded by ECM composition. Myoblasts rapidly respond to structural cues originating from topographical changes of the ECM backbone and micron-scale grooves between adjacent muscle fibers, through a mechanism known as contact guidance (Ippolito and Deshpande, 2023). This promises the generation of structurally sound muscle (Carnes and Pins, 2020; Browe and Freeman, 2019). The topographical cues allow for a consistently organized cytoskeletal profiles that are elongated parallel to the direction of the underlying nanotopography, and this occurs shortly after cell attachment (Fig. 2A). This is essential for the myoblast to remodel the

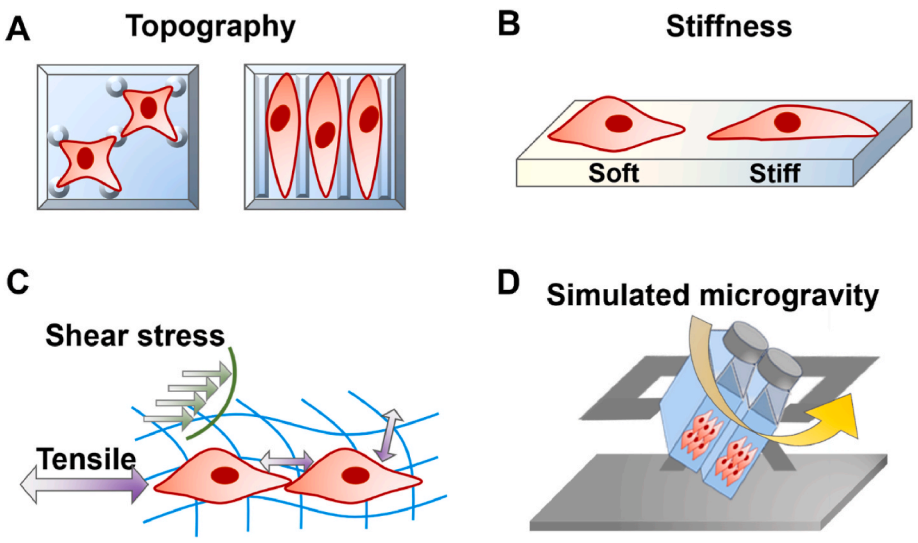

Fig. 2. Effects of mechanical cues on muscle cells, including Topographical feature (A), Substrate stiffness (B), Shear stress and tensile (C), and simulated microgravity (D).

anisotropic ECM by synthesizing and transferring ECM proteins (Jiao and Moerk, 2018).

Myoblasts require a certain level of surface roughness of matrix that allows their morphological function for the development of muscle fibers. The scale of irregularities can be classified into macro-roughness (100 μm to millimeters), micro-roughness (100 nm–100 μm), and nano-roughness (less than 100 nm), each with its preferential adsorption to specialized anchorage compounds of adhering cells (Dulgar-Tulloch et al., 2009). The high sensitivity of myoblast morphology to nano-scale roughness drives myotube organization for efficient contraction. In general, muscle regeneration involves the fusion of myoblasts to produce highly aligned multinucleated myotubes with diameters ranging from 20 to 100 μm (Kim et al., 2020). Highly oriented alignment of myotubes is prerequisite to generate maximal contractile force. The absence of structural cues failed to achieve unidirectional myotube alignment, resulting in a weak contractility of immature myotubes (Mackey et al., 2017). The pro-myogenic potential of myogenic cells can be enhanced by providing a dense structural and native-like microenvironment. Parallel alignment of fibers accelerates the homogeneously spatial distribution of cells, which is necessary for functional organization. Well-aligned cells contribute significantly to preserving the fiber's mechanical strength and stiffness, keeping it within the myogenic range (Young's modulus of 12 ± 1 kPa) (Ergene et al., 2020). These findings provided new insights into the potency of structural cues on controlling the myogenic cell fate.

3.2.2. *The ECM stiffness*

The inherent ability of SCs to affect and to acutely sense their ECM stiffness has been extensively demonstrated, which correlates with the varying degrees of cell shrinkage that occurs during specific stages of cell development (Fig. 2B). SCs are exposed to a temporally defined set of mechanical and biochemical signals from their surrounding microenvironment. The efficacy of cell response to their microenvironmental cues is mainly determined by the stiffness change over time. During the process of cytoskeleton elongation, cells exert traction forces, and also required appropriate substrate stiffness to withstand these forces. Scaffold stiffness correlated to its size/area reduction upon culturing conditions, suggesting different shrinkage degree by cell internal forces. Inhibition of scaffold shrinking by affixing device made of two Teflon sheets, which can be adjusted with screws and nuts (Levy-Mishali et al., 2009), ensures spacious cell organization with normal cell morphology. This indicate that environmental framework shrinkage can lead to cellular degeneration and shape deformation (Levy-Mishali et al., 2009). Dynamic alterations in the elasticity/stiffness and nanoscale roughness of the ECM result in cytoskeletal remodeling. This is accompanied by changes in cell shape from flattened to rounded, cells lose adhesion to the substrate (Rojas-Rodríguez et al., 2023).

Pliable substrates with a muscle-like stiffness with Young's modulus of 12 kPa are superior to conventional rigid plastic substrates for self-renewal of SCs(Gilbert et al., 2010). The propagation of primary myoblasts increased only on substrates with a specific stiffness with Young's modulus of 21 kPa, and was independent of varying protein coatings. However, the organization of myotubes with cross-striations and contractions are influenced by the combined effect of elasticity and protein coating (Boonen et al., 2009). Healthy muscles with soft stiffness (12 kPa) also promote myogenic progression. The introduction of RGD (Arg-Gly-Asp) adhesive peptides and soluble niche factors synergistically enables robust cell expansion. More compliant substrates are insufficient to withstand cell forces. Conversely, excessively firm matrix might not lead to parallel oriented myotube organization. Myogenic progression is halted on a hard substrate (40 kPa) irrespective of the uniform distribution of RGD peptides. The impaired cell viability caused by a relatively stiff substrates cannot be restored back to a healthy stiffness by subsequent substrate softening (Madl et al., 2021). This implies that by optimizing the stiffness of the scaffolds to withstand the cell forces, the effect of biochemical cues can be regulated. In addition, it is important to note that tissue is nonhomogeneous. There is compelling evidence indicating that substrate stiffness is a critical factor in the collective cellular dynamics of myotube alignment across multiple length scales. When cultured on mechanically anisotropic liquid crystalline polymer networks (LCNs), C2C12 myoblasts collectively polarize towards the stiffest direction of the substrate and actively remodel the ECM structure during the process of collective polarization. The alignment of three essential ECM proteins, fibronectin, laminin, and collagen IV, closely matched the corresponding myotube alignment in both magnitude and direction over time. These observations underscore the importance of stiffness anisotropy in orchestrating the global organization of myotube-ECM over millimeter-scale distances (Skillin et al., 2023).

3.2.3. *Tensile and fluid shear stress*

Muscle contraction and the movement of interstitial fluid within muscles results in mechanical loading of tensile and fluid shear stress (FSS) on the cell niches. FSS can be transmitted longitudinally or laterally from myoblasts to their surrounding endomysium and perimysium (Evertz et al., 2016) (Fig. 2C). This presents a possible

mechanism for protecting the underlying orientation of the ECM(Distler et al., 2020). Long chains of actin and myosin, called stretch-shortening filaments, generate longitudinal force that pass through the myotendinous junction in series. The varied contraction rate of different myofibers and neuro-vascular tracts of niches produce the local shear stress. Local shear distributions between neighboring myofibers synchronously exert the lateral force transmission to the ECM connected to myofibers (Haroon et al., 2021).

Following FSS-induced cellular deformation, the secretion of nitric oxide (NO) increases, which was shown to accelerate myoblast migration during the process of myoblast fusion with the myofiber (Haroon et al., 2022). Furthermore, FSS upregulates the expressions of cytokines that are essential for muscular repair, such as interleukin (IL)-6 and C-fos, which modulate the cell regenerative ability in autocrine or paracrine manners (Haroon et al., 2021). Remarkably, an important factor that protects the mechanoresponsive capacity of myoblasts to FSS is the ECM stiffness. Exposure to 1-h of pulsating FSS (3 Pa/s or 4 Pa/s, 1 Hz) increased myoblast proliferation on stiff substrates that simulated the rigidity of aged muscles (20 kPa), and this also increased the expression of C-fos and IL-6. In contrast, reduced myoblast proliferation was observed on soft matrices that reflected the stiffness of young muscles (0.5 kPa), as identified through the down-regulated expression of α-actin and myogenin (van Santen et al., 2022).

3.2.4. Gravity loading

Multicellular organisms have developed complex tissue mechanosensitive systems under the influence of Earth's gravity. Prolonged exposure to microgravity during long-term space flights can alter the mechanical homeostasis of tissues, leading to physiological changes such as muscle atrophy (Kim and Alcantara, 2023; Capri et al., 2023). The developing muscle cells inside the microenvironment can perceive and respond to gravitational loading, thereby contributing to the maintenance of muscle integrity and function (Andreeva et al., 2022). Investigations under spaceflight conditions, including both real and simulated microgravity studies, demonstrate the feasibility of using in vitro surrogate muscle protein constructs as a nutritional source for astronauts (Benjaminson et al., 2002). The available data shows reversible morphological and functional changes in cells exposed to microgravity. These perturbations are associated with the remodeling of cytoskeletal elements, the reorganization of microtubules and F-actin, and the expression of actin-related genes in a gravity-dependent manner (Andreeva et al., 2022). Several investigations conducted on simulated microgravity (Fig. 2D) have interpreted that the phenomenon of satellite cell degeneration is the main cause of muscle atrophy during spaceflight (Tarantino et al., 2020). Myoblasts experience aging due to dysfunction caused by microgravity. While a transient loss of mechanical loading can increase the number of newly elongated myotubes, prolonged simulated microgravity can lead to an inability of myotube differentiation (Takahashi et al., 2021). The structured ECM acts as an important gravisensitive unit component, complementing graviperception in facilitating cell physiological adaptation to microgravity. However, questions remain regarding the changes in the major mechanical properties of the existing ECM and the de novo synthesized matrix upon gravity deprivation (Andreeva et al., 2022).

3.3. The architectural basis of cell mechanics in myogenesis

Mechanical model of cell structure has been proposed, which posits that the entire cell is a prestressed tensegrity architecture. In the model, tensional forces are borne by cytoskeletal microfilaments and intermediate filaments, and these forces are balanced by interconnected structural elements that resist compression, most notably, internal microtubule struts and ECM adhesions (Ingber and Tensegrity, 2003). Tension elements, such as elastin and reticulin fibrils, concatenate collagen fibers that act as compression resistance elements on the ground substance. During the process of mechanotransduction, transmembrane focal adhesion (FA) complexes are recognized as the principal element that bridge the interaction of the extracellular and intracellular tensegrity, and perceive and transmit mechanical signals from the ECM to the cytoskeleton (Andreeva et al., 2022). The formation of this complex occurs when cells adhere to the ECM on the cytoplasmic side of the plasma membrane at sites of ECM attachment. The actin cytoskeleton then directly receives and responds to external mechanical stimuli perceived by FA complexes (Argentati et al., 2019). Specialized molecular hubs then convert these stimuli from the extracellular environment to the nuclear lamina, nucleoskeleton, and intra-nuclear compartments (Andreeva et al., 2022). The structural links of the three major types of cytoskeleton filaments: actin filaments (F-actin); microtubules (MTs); and intermediate filaments (IFs), can stabilize the cytoskeletal mechano-sensitivity of muscle cell precursors (Jabre et al., 2021). MTs and actin fibers are tightly interconnected with cross-linked IF bundles, forming a rigid framework. This framework ensures cytoskeleton plasticity when the external environment is mechanically altered (Andreeva et al., 2022). As the endpoint of cytoskeletal force transmission from cell periphery to the nucleus, the nucleoskeleton is integrated into the cell cytoskeleton via the Linker of Nucleoskeleton and Cytoskeleton (LINC) complex. During the process of mechano-responses in myogenic cells, these cytoskeleton components undergo significant reorganization and each nucleus is spatially redistributed towards the myofiber periphery (Iyer et al., 2021) (Fig. 3).

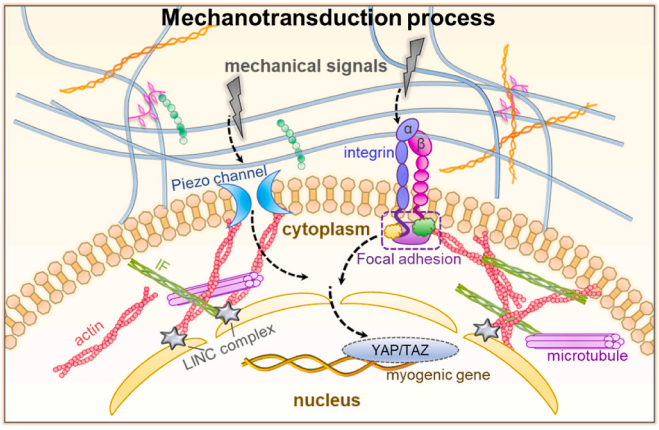

Fig. 3. Cytoskeleton components and molecular mechanism involved in the mechanotransduction process. Mechano-sensors, such as cell-surface integrins and Piezo channels respond to the extracellular mechanical signals, and initiate the movement and reorganization of the cytoskeleton filaments. These mechanical changes activate the biochemical signaling cascades that regulate downstream gene expression and contribute to myogenesis. Following the initial fusion of elongating myoblasts into long linear myotubes, the actin network undergoes remodeling, with the tensive fluctuation of the actomyosin complex (Bruyère et al., 2019). Membrane fluctuations and tension function act antagonistically, forming a thermodynamic barrier that mediates membrane fusion. Differentiated cells exhibit higher membrane surface fluctuation-tension than their origin/precursor cells, which ensures their maintenance of terminal differentiation state. Artificial coupling of stressed or tensed stimuli to membranes could enhance fusion efficiency (Chakraborty et al., 2022). In mature myotubes, stiff MTs networks show high-density and a more ordered paraxial array, which may maintain cell morphology (Wang et al., 2015; Pegoraro et al., 2017). The softness of IFs allows cytoskeletons to adapt and resist large deformations, up to 300% of their initial size (1–3 μm) (Block et al., 2015). On account of the mechanical interconnections of these major cytoskeleton filaments to one another, to membrane sensors, and to organelles, the multinucleated myofibers provide support for muscle elasticity, plasticity, and resistance to physical force loading. Simultaneously, the mechanical movement of these cytoskeleton filaments can trigger changes in the transcriptional events, thereby regulating cell fate determination (Uroz et al., 2018).

4. Engineering hierarchically structured CM

Integrated muscle fibers containing the appropriate amounts of myoglobin, actin, and myosin, are necessary to achieve the desired organoleptic features, including toughness and texture of meat (Fish et al., 2020). Cultivating cells in a suitable 3D scaffold can induce the formation of tissue structures with enhanced mechanical properties (Bomkamp et al., 2022). Hydrocolloids represent a heterogeneous group of polymeric materials that are readily dispersed in water, exhibiting a tendency to swell in water. Hydrocolloids mainly contain polysaccharides and some proteins, are primarily composed of polysaccharides and some proteins, which serve as thickeners, gelling agents, and emulsion stabilizers, thereby enhancing the rheological and textural properties of food products (Pirsa and Hafezi, 2023). Hydrocolloids-based scaffold engineering typically aims to enhance the reparative competency of grafts or post-transplantation robustness of implants for biomedical applications, but there are an increasing number of works that have applied it to the enhancement of CM quality. Indeed, sustainable scaffold innovations are being developed for various purposes other than biomedical applications, including the use of structurally multifarious hydrocolloids for mechanical regulation in the production of engineered CM.

4.1. Hydrocolloids-based biomaterials for engineered structured scaffolds

Effective scaffolds for CM should afford controllable mechanical elements to induce the formation of organized tissue. Since the scaffold is a crucial component of artificial cellular microenvironment, it is highly desired to replicate natural ECMs, from the structural and mechanical perspective (Xu et al., 2015). To enable contact guidance, a positively charged and hydrophilic surface is required. Additionally, the substrate surface should include cell adhesion and proteolytic sites to facilitate cell adhesion and migration. For instance, scaffold surfaces should always present cell recognition moieties such as RGD peptide motifs to enhance the molecular bonds formed with mechano-sensors on cell membranes, thereby promoting cell adhesion (Ng and Kurisawa, 2021). Synthetic biopolymers have molecular structures that are advantageous for easy functionalization and intrinsic polymerization. However, their hydrophobic nature and lack of cell recognition sites limit their applications for CM(Singh et al., 2023b). Inedible synthetic polymers and polymer leaching treatments to dissociate cells prior to consumption should be avoided. Numerous hydrocolloids have been identified that can create new possibilities for feasible, low cost and scalable solutions. Most hydrocolloids are plant-derived, including both terrestrial plant and seaweeds. Animal-derived and microbial proteins and polysaccharide can also exhibit structural functions to define the texture (in-product and in-mouth) and sensory properties of newly fabricated foods (Manzoor et al., 2020). Scaffold fabrication can benefit from the potential formulations that form the basis of colloid component interactions and finely assembled design. Hydrocolloids, including collagen, gelatin, gelatin methacrylate (GelMA) and fibronectin (FN), have the capacity to self-assemble into 3D scaffolds with certain mechanical characteristics. These characteristics support the growth of bovine and rabbit myoblast cells (MacQueen et al., 2019; Furuhashi and Morimoto, 2021a; Ahmad et al., 2021). As major structural proteins, collagen, FN, and laminin have advantages in promoting cell spreading and differentiation. Animal protein scaffolds are considered the gold standard and include mainly gelatin and collagen scaffolds. However, the mechanical stability of these self-assembled networks may decrease during processing (Singh et al., 2023a; Seah et al., 2022b). The compounded colloidal systems composed of collagen or gelatin, and anionic polysaccharides (e.g., alginate, chitosan) provide another strategy that benefits physical performance more than their individual components (Enrione et al., 2017). However, there are certain drawbacks due to the high production cost of animal-derived proteins, which makes them unavailable for large-scale application and sustainable development of CM. Plant-based hydrocolloids have rightfully garnered considerable attention, due to their intrinsic qualities of being edible, and environmentally friendly. Polysaccharide isolated from plants (e.g., cellulose, Starch, konjac) show good gel-forming property, high stability, bioactivity, and good mechanical strength (Singh et al., 2023b). The natural biocompatibility and aqueous stability of plant-based proteins (e.g., soy protein (Ben-Arye et al., 2020; Wei et al., 2023), prolamin (Su and Jing, 2023), peanut protein (Zheng et al., 2022a, 2022b), and hypoallergic pea-protein (Ianovici et al., 2022)) make them prime choices for vegetarian alternatives to meat and for the production of porous scaffolds in the form of sponges, fibers, or meshes. These scaffolds are generally fabricated through physical treatments that involve wiredrawing, thermal heating, and hydrostatic pressure (Zheng et al., 2022a). Natural cross-linking agents, such as microbial transglutaminase, act as green catalysts to promote the formation of strong covalent bonds. Consequently, they can effectively modify the gel strength, WHC, and viscoelasticity (Jahangirian et al., 2019). Recent advancements in plant protein bioink design have led to the development of various 3D-printable scaffolds with thick and complex structures. Despite the strategically restrictions that remain a major challenge in structural regulation and stabilization, scaffolds derived from plant proteins are strong substitute candidates for mimicking the ECM during cell seeding and growth support.

Biopolymers, such as hyaluronic acid (HA), chitosan, and pullulan, generated using endotoxin-free microorganisms (MOs), can serve as alternative sources for structured scaffold fabrication (Pajčin et al., 2022). Apart from this, microbial proteins have recently been used to develop novel edible CM scaffolds due to their production process not being limited by agricultural techniques or environmental factors, unlike animal and plant proteins. Probiotic bacteria have also been incorporated into renewable scaffolding materials because of their ability to produce antimicrobial substances, hydrogels, and fibril scaffolds (Kolodkin-Gal et al., 2023). Yeast proteins (YPs) that have a healthy balance of amino acids and are lower allergenic (almost no allergens), making them an attractive alternate of traditional protein sources. Therefore, it could partially replace animal-derived proteins and produce hypoallergenic, odorless, and highly nutritious products that meet the demands of biocompatibility and mechanical properties (Wang et al., 2024).

4.2. Engineering CM with multiple types of scaffolds and fabrication techniques

Various forms of scaffolds are available for CM engineering, including microcarriers (MCs) for large-scale cell expansion, hydrogels, porous or fiber scaffolds, decellularized scaffolds, and edible films for tissue maturation (Table 1). These scaffolds have been given specific physicochemical characteristics, mechanical strength, and surface functionality through bioengineering technologies (Seah et al., 2022c). Scaffolds with sponge-like and fibrous structures contain interpenetrating polymer networks that facilitate the circulation of micronutrients and gas. Fiber scaffolds, which exhibit morphological similarities to natural muscle tissue, are effective in promoting cell alignment through contact guidance. Hydrogels are cross-linked using hydrophilic polymers to replicate the large WHC of naturally occurring ECM. The combination of cytocompatible materials and a non-toxic cross-linking process creates an optimal microenvironment for embedding and supporting cells. Decellularized scaffolds, mainly derived from decellularized plant leaves, retain their natural striated geography, making them suitable for cell-matrix attachment and myofiber alignment. Edible films created using micromolding technology offer a controllable platform for inducing final myogenic differentiation and forming oriented parallel muscle fiber morphology (Orellana et al., 2020a).

Table 1
Examples of food hydrocolloids-based scaffolds and their mechanical parameters.

Scaffold types	Examples	Fabrication methods	Remarks
Microcarriers	Grooved gelatin MCs (Norris et al., 2022)	Emulsion-templating and embossing	• Easy to implement based on the water-in-oil emulsion • Tunable stiffness based on gelatin concentration in aqueous phase • Predefined grooved striations that guide cell alignment and aggregates for microtissue formation
	Chitosan-collagen MCs (Yen and Glusac, 2023)	Electrospray	• A softer texture • Burger-like appearance and layered cultured meat by aggregating • MC-derived microtissues and oleogel-based fat substitutes
	UVA-riboflavin/eggshell membranes collagen MCs (Andreassen et al., 2022)	Lyophilization	• Low cytotoxicity • Resistance to degradation in stirred bioreactor • Increased surface area and mechanical strength • Significant value in by-products resource availability
	Gelatin MCs (Liu et al., 2022)	Lyophilization	• From commercialized 3D TableTrix tablets • Microtissue modular for 3D-printed assembly of meatball analogs
Porous scaffolds	pea/soy protein scaffolds (Ianovici et al., 2022)	3D bioprinting	• RGD modified • High fluid absorption for nutrient and oxygen delivery • Elasticity close to Young's modulus (~8 kPa) for the native muscle tissue of a young bull
	Salmon gelatin/sodium alginate scaffolds (Enrione et al., 2017)	Lyophilization	• Non-mammalian source • Adjustable stiffness
	Textured soy protein scaffolds (Ben-Arye et al., 2020)	3D bioprinting	• Enable co-culturing of tissue-specialized cells of muscle and connective tissue • Improve flavor, sensorial perception, and texture
	Soda bread crumb (Holmes et al., 2022)	Existing food fabrication	• Recipe for bread • Eliminate the steps of decellularizing and purifying plant proteins or decellularizing tissue
Hydrogels	Plant/insect protein hydrolysate hydrogels (Dutta et al., 2022)	3D Bioprinting	• Strong antioxidative properties and mechanical strength • Enhance myogenesis under serum-reduced conditions • Enhance the early myogenic differentiation of bovine muscle SCs
	GelMA hydrogels (Jeong et al., 2022)	3D bioprinting	• Precise printing of geometrically structures • Enable both myogenesis and adipogenesis while controlling the muscle-to-fat ratio
	GelMA-silk fibroin hydrogels (Li et al., 2021)	3D bioprinting	• Stable interwoven lattice structures • Create multi-layered myofibers that encase stretched, and extensively fused multinucleated myotubes
	Collagen/fibrin-matrigel hydrogels (Furuhashi and Morimoto, 2021b)	Hydrogel casting	• Myocyte-laden modules with stripe micropatterns • Can be electrically exerted to generate contractile myotubes • Contractile muscle constructs can be enlarged in size by stacking the modules
	Polysaccharide-pea/soy protein hydrogels (Wollschlaeger et al., 2022)	Molecular assembly	• Homogeneous cell-laden • Biocompatible temperature range for solution-gelation transition
Fibrous scaffolds	Alginate core-shell structured microfibers (Ding et al., 2023)	Microfluidic printing	• Soft cell-laden hydrogel core • Induce the high differentiation and maturity of porcine muscle stem cells (PMSCs), forming spontaneous contraction on the macro level
	Soy protein amyloid fibril scaffold (Wei et al., 2023)	Molecular assembly	• Stable sheet structure
	Wheat glutenin fibrous scaffolds (Xiang et al., 2022a)	Molecular assembly and ice-templating	• Aligned sheet structures • Facilitate cells elongate along the sheet orientation, and grow into densely packed striated fibers with ECM deposition
	Zein-hordein scaffolds zein-secalin scaffolds (Su and Jing, 2023)	3D bioprinting	• Tessellated morphology with a pore size of 400 μm • Triggers muscle cells to form spontaneously into a meat-like slice over the course of 1 week
	Peanut wire-drawing protein scaffolds (Zheng et al., 2022b)	Extrusion	• Stimulate cells to secrete ECM proteins such as collagen I and collagen III
Nanofibers	Cellulose acetate @ annatto nanofibers (Dos Santos et al., 2023)	Electrospinning	• Favor a long-term proliferative state of muscle cells
	Gelatin fibers (MacQueen et al., 2019)	Immersion rotary jet spinning	• Comparable fiber diameters to natural collagen fibers • Controllable fiber size promotes cell aggregation or alignment
Decellularized scaffolds	Green onion-derived cellulose (Cheng et al., 2020)	Decellularization	• Repeating groove with 20 μm in width, 10 μm in depth, and 5 μm in separation • Promote myoblast alignment and their fusion into myotubes
	Decellularized ECM from bovine fibroblasts monolayer (Lee and Jackson, 2023)	Decellularization	• Includes ECM proteins and growth factors • Amplifies cell numbers by up to 500-folds • Maintains the differentiation abilities of stromal cells from bovine umbilical cord under serum-reduced conditions
Edible films	Chitosan-carboxymethylcellulose (CMC) porous multilayer film (Park et al., 2021)	Molecular assembly	• Capability to release C-PC • Enhance myoblast proliferation under serum-reduced conditions
	Salmon gelatin-based micropatterned film (Jaques et al., 2021)	Cold casting and molecular assembly	• Microchannel with a width, depth, and separation of 100 μm each • Furnish muscle cell organization with a fiber-like morphology
	Collagen-based composite films (Wang et al., 2023)	Molecular reassembly	• Proanthocyanidins and dialdehyde chitosan stabilize the self-assembly and triple helical structure of collagen molecules • Good thermal performance • Macro-roughness facilitates the cell dispersion, and the cell cycling progression toward myogenic differentiation

4.2.1. MCs

MCs are polymeric beads with diameters typically less than 500 μm. Scaffolds have significant implications for the scalability and cost-competitiveness of CM by enabling the transition of anchorage-dependent mammalian cells to MC-based suspension bioreactors (Verbruggen et al., 2018). MCs developed for muscle stem cells and meat production are expected be embedded in the final product and therefore need to be edible. They may also act as a temporary substrate for cell proliferation but must be degraded during the bioprocess (Bodiou et al., 2020). Compared to stationary culture, the cultivation of MC in a dynamically mixed bioreactor, such as a stirred-tank bioreactor (STR), fluidized-bed reactor (FBR), or rocking-bed reactor (RBR), increases the surface area for improved diffusion of media and gas into the microspheres (A 30% diameter penetration results in 70% of the volume being

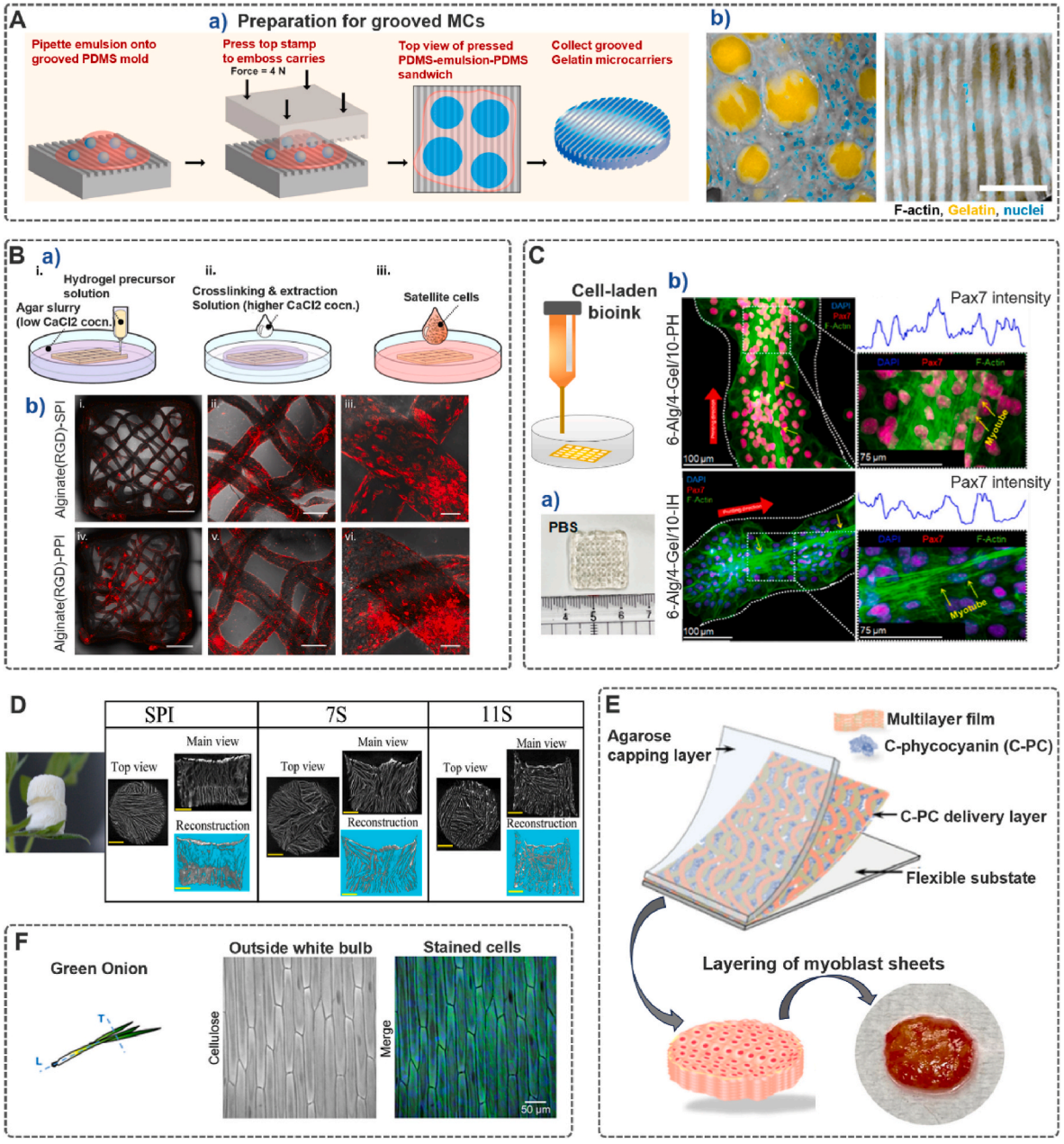

Fig. 4. Examples of multitype scaffolds for structured CM. A. Edible grooved gelatin MCs. a) Formation of grooved microstructures by dropping the emulsion onto a grooved PDMS stamp. b) Confocal images of expanding C2C12 cells seeded on spherical MCs (left) and grooved MCs (right) at 7 days. Scale bar: 500 μm. Copyright (2022), with permission from Elsevier (Norris et al., 2022). B. 3D-printable plant protein scaffolds. a) Schematic of the 3D printing process for SPI/PPI scaffolds. b) Confocal images of spreading BSCs on plant protein scaffolds. SPI: soy isolate protein. PPI: pea isolate protein. Scale bars: 2 mm (i&iv), 500 μm (ii&v) and 100 μm (iii&vi). Copyright (2022), with permission from Elsevier (Ianovici et al., 2022). C. Hydrogels from plant/insect protein hydrolysates. a) The geometry of 3D-printed constructs. b) The localization of Pax7 (red) in nuclear (blue) signifies the early myogenesis within hydrolysate-enriched hydrogels. Adapted with permission from (Dutta et al., 2022). Copyright (2022), American Chemical Society. D. Soy protein amyloid fibril scaffolds. Macroscopic morphology (left) and structural characterization of scaffolds (right). Scale bars: 2 mm. Adapted with permission from (Wei et al., 2023). Copyright (2023), American Chemical Society. E. C-PC released porous multilayer film. a) The structure characteristics of porous films. b) Fresh mold of a multilayered CM. Adapted with permission from (Park et al., 2021) Copyright (2021), American Chemical Society. F. Green onion cellulose scaffolds. Confocal images reflect the highly uniform structure originating from the outer white bulb (left, gray) and aligned myotubes within the inner grooves (right, green F-actin and blue nuclei). Adapted with permission from (Cheng et al., 2020) Copyright (2020), American Chemical Society.

available for nutrient diffusion). Agitated systems permit nutrient feeding with a high rate whilst also protecting cells within the MCs against mechanical damage, shear forces caused by liquids, and bead-bead collisions (Derakhti et al., 2019). Agitation rate and technical feasibility of stirred-system are the most important factors in mass and energy transfer to achieve the pre-defined goal (Pajčin et al., 2022). Phase separation is a commonly used technique for MC preparation. A previous study has described the use of poly (lactic-co-glycolic acid) (PLGA) microspheres, prepared by thermally induced phase separation (TIPS) technique, for expanding smooth muscle cells (Ahmadi et al., 2011). Electrified liquid jets (Jiao et al., 2019; Zernov et al., 2022) and embossing processes (Norris et al., 2022) (Fig. 4A) have been considered to offer differently sized and textured microspheres that promote mechanical qualities. Tunable surface topologies and stiffness of MCs have been enabled by combining an imprinting technique with emulsion-templated microparticles, as recently proposed by Norris et al. Novel textured gelatin microspheres have been engineered to mimic the native striated architecture of the mechanical microenvironment of skeletal muscle, which have been demonstrated to possess the capacity to promote the growth and alignment of myogenic cells. The proliferating cells and MCs with a Young's modulus of 14 kPa then aggregated into a meat analogue that exhibited a stabilized structure during heating (Norris et al., 2022).

4.2.2. Cytocompatible hydrogels

Hydrogels are a rational choice for reconstructing 3D-like microenvironment for three main reasons: (a) cells can be embedded by the hydrogels and are supported by the 3D ECM-mimetic microenvironment. Preferably, hydrogels can be degraded by cells over a culture period, allowing for the synthesis of nascent ECM components; (b) the excellent WHC allows a suitable diffusion kinetics by which nutrients can penetrate the entire thickness of the microenvironment (Bomkamp and Skaalure, 2022); and (c) the capabilities for growth factor-binding and loading can provide optimal feasibility of biochemical cues due to the localized and sustained-release of growth factors. Cell-laden hydrogels with tunable mechanics and surface topology can be prepared as structural modules used in the accumulation of multilayered organization. A recently established method by Furuhashi et al., wherein polydimethylsiloxane (PDMS) hydrogels were fabricated, resulted in striped structures that were alone sufficient to guide cell alignment. Subsequently, millimeter-thick muscle tissues with densely distributed and contractile myotubes were generated by fusing stacked hydrogels loaded with bovine myoblasts (Furuhashi and Morimoto, 2021b). Thus, hydrogels are at the forefront of the scaffolding systems proposed to be promising candidates to reproduce CM with realistic textural properties.

Hydrogels crosslinked by structural ECM proteins, such as collagens, help to substantially maintain the adhesive and constructive backbone for the 3D-embedded myoblasts to generate aligned and large multinucleated myotubes (Prüller et al., 2018). Gelatin-based hydrogels can fulfill the structural and biochemical feature requirements of natural tissues while beingless immunogenic than collagen (Yang et al., 2015). Gelatin offers various functional groups such as aldehydes and aspartic and glutamic acids, which can be used for chemical crosslinking methods. Heating and irradiation processes are also commonly used for physical crosslinking operations (Rao et al., 2023). Additionally, gelatin can be readily molded into the requisite microchannel geometries for microfluidic cell culture or multi-channel hydrogel filaments, thereby providing a topographical microenvironment. This is essential for the alignment of myoblasts, the formation of myofibers and the development of pre-vascularized muscle-like tissues (Chen, 2020; Bolívar-Monsalve and Ceballos-González, 2022). Therefore, gelatin-based hydrogels show particular promise and benefits for generating an artificial ECM that more closely mimics the in vivo microenvironment.

4.2.3. Microfiber and nanofiber scaffolds

In light of the morphological architecture of well-organized myofibers in natural muscle tissue, scaffolds with aligned fibrous sheets have been identified as a promising avenue for enhancing the myogenic potential of adhered cells. Common methods to reconstruct the topological resemblance to the native fibrous basal layer are micropatterning and electrospinning. Micropatterning techniques are used to create 2D fibrous channel walls such as uniaxial basal lamina of aligned myofibers along the main axis of the muscle tissue (Narayanan et al., 2020a). However, uniaxial channels are inadequate for imitating the anisotropic mechanical structures found in dense connective tissues, which are composed of and a random collagen fibril network interspersed with aligned ECM fibrils. The progress made in the fabrication of 3D nanofiber scaffolds have resulted in improved textural properties of products on account of their ECM-mimetic architecture (Uehara et al., 2020). Extensive efforts have been devoted to exploring versatile nanofiber materials, ranging from 3D nanofiber aerogels/scaffolds, to those prepared by direct electrospinning, 3D bioprinting, microfluidic platforms, and self-assembly of short nanofibers into nanofiber microspheres (Ding et al., 2023; Chen et al., 2020; Su et al., 2023). Due to inadequate porosity and inaccurate spatial control in nanofibers, cells adhere primarily to the outside of the scaffolds. This can result in regions of acellularity. Solution-electrospinning techniques, by which cells are encapsulated and electrospun into micro/nanofibers can help to overcome these limitations (Yeo and Kim, 2018). Attempts to engineer functional 3D organization using aligned myoblast-seeded bundles, such as a fibrin/polyethylene glycol (PEG) microfiber bundles loaded with C2C12 cells were developed by the Guo group. The fabricated microfiber bundle scaffolds showed excellent properties for myogenically induced cells to proliferate, elongate and become multinucleated myotubes (Guo et al., 2019). The focus of high throughput production of electrospun nanofibers is to develop processes that achieve greater scalability. Certain natural materials with highly oriented structures may offer desirable topological characteristics similar to those produced by spinning techniques. For instance, mycelial mats from certain fungal and algae species provide inspiration for the manufacture of fibrous nanostructures with an average diameter of less than 5 μm (Narayanan et al., 2020b).

4.2.4. Decellularized plant scaffolds

Decellularization, which aims to retain the biological architecture of the ECM after cell removal, has been an accessible alternative for partial recapitulation of the 3D machinal microenvironment. The cells are removed by detergents such as Triton X-100 and sodium dodecyl sulfate (SDS), but cellulose structures are usually preserved in decellularized tissues (Gilpin and Yang, 2017). Decellularized ECM (dECM)-derived hydrogels have been shown to promote cell growth and orientation through tissue-specific biochemical components and topographical cues that align myoblasts, resulting in the efficient development of thick and elongated myotubes (Kim et al., 2020). However, the use of animal-derived scaffolds is not recommended due to sustainability concerns. Natural cellulose sources, such as plants, algae, and bacteria have been developed as scaffolds with appropriate biocompatibility, biodegradability, and low immunogenicity (Modulevsky et al., 2014; Fontana et al., 2017). The pore diameters, aspect ratios, and degrees of anisotropy of decellularized structures of cellulose from common fruits and vegetables are desirable for osteogenic or myogenic stimulation, prior to the formation of connective tissue or organized muscle, respectively (Ng and Kurisawa, 2021; Salehi et al., 2021). Scaffolds derived from carrot, broccoli, cucumber, and potato cellulose exhibited various interwoven circular structures with different sizes, favorable for the arrangement of human and mouse skeletal muscle cells (Contessi Negrini et al., 2020; Lu et al., 2022a). Apple-based cellulose scaffolds displayed tunable surface biochemistry (Modulevsky et al., 2014). Interestingly, a hydrogel with ECM adhesion motifs can be casted onto the scaffolds to significantly improve the cell invasion. Such composite biomaterials provide an eco-friendly strategy to customize the shape and microenvironment of scaffolds. A distinct section of the green onion

possesses highly uniform surface micropatterning that could be exploited to determine the directionality of cell alignment. In particular, the well-ordered grooves of the outer white bulb segments are characterized by grooves of 20–30 μm width and less than 10 μm depth. It provides a unique topographical microenvironment that is essential for uniaxial cell alignment and myotube fusion (Lu et al., 2022b).

4.2.5. Edible films

Following the applications of film scaffolds in tissue engineering, films derived from natural sources (e.g. gelatin, plant-based proteins, cellulose, alginate, konjac, chitosan) are currently emerging with the goal of edibility and biodegradability. The morphological features should be considered first when designing films for CM; the oriented microstructure is a key factor in facilitating cell orientations conducive to myogenic processes. Casting of prepared solutions onto tailor-made micropatterned substrates is the most common option for structuring the films, and this is proven and effective to regulate bovine myoblast orientation and supported their growth into fiber-like morphology. Compared to flat featureless films, which produced unorganized cell monolayers, the formation of fiber structures on oriented films was evidence that parallel microgrooves prompted cell alignment. This enabled the successful cultivation of tissue with a fiber-like morphology from muscle cells (Orellana et al., 2020b). Accordingly, Xiang et al. replicated the parallel microgroove of polydimethylsiloxane (PDMS) molds on protein-/polysaccharide-based films, in which the pattern dimensions range from 110 to 3800 nm in depth and 1–100 μm in width. The films made of protein and polysaccharide exhibit distinct topography and roughness characteristics. The protein films display relatively smooth fractures along cross-sectional surfaces, while the polysaccharide films have coarse cross-sectional morphologies. Additionally, plant protein films have advantages in promoting cell adhesion, particularly soy, zein, and glutenin protein-based films, indicating the most promise for CM engineering based on mechanical strength (Young's modulus above 200 kPa) and material availability. The patterning on these films allowed for higher tensile strain in the stretching direction. The patterned zein films exhibited enough mechanical strength (Young's modulus above 4000 kPa) to support myotube formation while also increasing the degree of chewiness (Xiang et al., 2022b).

5. Prospects and challenges

Scaffolds that imitate the vital mechanical and biochemical attributes of the muscle tissue microenvironment have emerged as having significant pertinence in the manufacturing of structured CM with desirable textural properties. A key challenge exists in how to address the mechanistic context of mechanically controlled myogenic differentiation and organization. Regenerating muscle is made up of various mononuclear cells and myofibers that have transcriptionally heterogeneous nuclei. Single-nucleus RNA sequencing (snRNA-seq) and spatial transcriptomics have started to reveal the cell-fate heterogeneity at subcellular resolution (Petrany et al., 2020). The identification of specialized cell population subtypes that can respond to the mechanical microenvironment are expected to provide valuable clues for how to develop and exploit suitable scaffolds and cell seeds in efforts to produce highly vascularized and organized muscle tissue structures.

Structured scaffolds physically underpin the production of CM that possess certain thicknesses and mechanical properties. Recent in vitro studies aim to replicate the in vivo microenvironment, specifically the biophysical properties of the structured ECM. Stiffness-controllable substrates have been developed to investigate the effect of stiffness on cell behavior. However, most of these studies have focused on producing uniform stiffness on a single substrate, and there are limitations in replicating the gradient changes of ECM stiffness. This impedes the identification of critical stiffness that affects cell-ECM interaction, and the remodeling of spatiotemporal heterogeneous mechanical microenvironments in both physiological and pathological conditions (Zhu et al., 2021). Microengineering structurization technologies, such as 3D printing and soft lithography combined with photopolymerization, have successfully achieved aligned topography and biomolecular gradients in a flexible and robust manner. For instance, hydrogels with biomolecular gradients immobilized on aligned topography can serve as multidimensional cell culture platforms to further engineer biological gradients (Shi et al., 2023; Wang et al., 2016). It should be mentioned that the production process of CM should aim to be cost-effective and efficient, including the techniques used for scaffold generation and structuring. Freeze structuring represents a promising approach to introduce aligned lamellar structure into 3D porous architecture (Seah et al., 2022a). By modulating the temperature gradient during in situ freezing procedures, it is feasible to generate a gradient structure with anisotropic pores (Zhang et al., 2017). This gradient scaffolds resulted in the self-seeding of cells, which was attributed to the spontaneous capillary effect induced by the gradient channel structure (Li et al., 2019). One potential future direction is to improve control over the porous and lamellar structure in food materials.

Future research could focus on determining the appropriability of utilizing engineered plant-derived biopolymers in realizing digestibility, feasibility, and industrial scalability of scaffolds, rather than solely relying on their conceptual appeal. Derived from extensive research on cell-cell and cell-microenvironment interactions, technologies for 3D co-cultured scaffolds have become attractive for the co-culturing of myoblasts and adipocytes. The precise regulation of adipogenesis presents an additional challenge in creating desired fat content, marbling, and softness attributes in CM. Novel manufacturing techniques are also expected to improve scaffold plasticity through the implementation of electrical stimulation (Nagamine et al., 2018), mechanical stretching (Haroon et al., 2021)and compression (Tao et al., 2023; Shahin--Shamsabadi and Selvaganapathy, 2022). The principles of cellular self-organization enable the formation of region-specific organoids. Structured CM can also be fused with muscle, adipose, and vascular organoids (Vogt, 2021). These innovations and their combination in CM engineering may yet reveal other beneficial effects of their final CM products.

6. Conclusions

Lab-grown technology has made CM a potential solution to meet the rising demand for eco-friendly meat supply systems. To achieve the desired toughness, structure, and texture for meat, mature and structured muscle tissue containing the appropriate amounts of myoglobin, actin, and myosin is required. The production of CM begins with myogenic or adipogenic cells that are isolated from animal tissue and have the ability to regenerate in vitro. Therefore, a deeper understanding of the growth environment of animal muscle tissue can improve the texture and simulate the palatability of traditional meat more effectively. Cell-extrinsic signals, particularly mechanical cues, from the niche and cellular microenvironment have been emphasized in muscle tissue engineering for biomedical applications. The lack of sufficient 3D mechanical support can result in poorly organized muscle tissue due to a reduced ability to control cell distribution and development. However, the significant value of mechanical microenvironmental cues for sustainable scaffold innovation and CM research needs to be thoroughly discussed.

In recent years, related studies have accumulated novel perspectives on the mechanical microenvironment that modulates myogenic differentiation. The inherent mechanical properties of the ECM, such as its topographical and geometrical characteristics and stiffness, as well as the external forces exerted on or delivered through the ECM, such as tensile, fluid shear stress, and gravity loading are highly orchestrated to contribute to the growth and maturation of ordered multinucleated myotubes. The mechanism for contact guidance, which is based on cell-ECM interactions, enables cells to respond sensitively to mechanical signals from their surrounding microenvironment. Topographical cues

work together with ECM stiffness to synergistically activate downstream signaling, ultimately controlling cell morphology, proliferation, and differentiation. Muscle fiber orientation and spatial heterogeneity contribute significantly to maintaining tissue mechanical strength within the myogenic range. Micron-scale grooves between adjacent muscle fibers and the surface morphology of the ECM guide rapid cell distribution and cytoskeletal morphology. This occurs shortly after cell attachment, a prerequisite for fusion of migrating myoblasts and remodeling of the anisotropic ECM structure. ECM stiffness can also be sensed by surrounding cells to withstand the varying degrees of cell shrinkage that occur during the process of cytoskeletal elongation and subsequent stages of myogenic differentiation. Muscle-specific stiffness with a Young's modulus of 12 kPa is essential for successful myogenic progression, which may be irreversibly compromised by the increased Young's modulus of 40 kPa. Anisotropic stiffness is an important regulator of collective cellular dynamics during cell polarization across multiple length scales, thereby controlling the alignment of ECM structural proteins corresponding to myotube alignment. In addition, changes in substrate stiffness influence the mechanoresponsive capacity of cells exposed to mechanical forces such as tension and FSS, which result from muscle contraction and movement of interstitial fluid within muscles. Fundamental morphological and functional attributions in muscle cells require the loading of Earth's gravity. Changes in the physiological behavior of cells have been shown to respond and adapt to microgravity, such as the inability of myotubes to differentiate. The biomechanical basis of the cell itself in myogenesis relies on the main extracellular and intracellular mechanical elements that play a fundamental role in force sensing. Together with protrusion extension and cell body retraction, interaction with the substrate via specific FA complexes has long been considered an essential step in driving downstream biochemical cascades. Mechanosensitive molecular nodes, including cell surface integrins and piezo channels, are highly activated and function to maintain the muscle cell in response to mechanical stimuli. The cytoskeletal mechanosensitivity of muscle cell progenitors depends mainly on the synthesis and reorganization of intracellular structural links of F-actin, MTs and IFs, which bridge membrane sensors and organelles. The mechanical interconnections of cytoskeletal filaments promise to provide cells with the necessary mechanical plasticity to maintain their morphology and structure as they adapt and resist large deformations under mechanical stress.

Efforts have been made to improve scaffold performance for better cell proliferation, differentiation, and tissue development. Innovations should ideally improve desirable organoleptic properties from a structural and mechanical perspective. Scaffolds mimic the ECM for cell attachment, and allow for precise spatial tuning and structural replication of the 3D mechanical microenvironment. In order for CM to achieve mechanical properties similar to conventional meat, a variety of scaffold forms are used, typically including MCs, hydrogels, porous or fiber scaffolds, and edible films for tissue maturation. The major challenge is designing a scaffold that is food-grade for use in the final product. Decellularized ECM is a promising category of potential scaffolding materials due to its inherently native-tissue geography and structure. It is important to pay attention to the combination with food-safe decellularization techniques.

Currently, synthetic materials are not suitable for use as CM scaffolds intended to produce safe-to-consume products. This is primarily due to the requirement for scaffold materials to either be appropriate for direct consumption or biodegrade rapidly. Unless new innovations can address these challenges, the use of synthetic materials as CM scaffolds will remain limited. Hydrocolloids-based biomaterials, particularly naturally derived hydrocolloids, are being developed to engineer structured scaffolds sustainably using the principles of colloid component interactions and finely assembled design. It is important to consider plant-derived sources as they align with the vision of CM. Plant-based hydrocolloids can be classified into two categories: proteins, such as soy protein, prolamin, Zein, peanut protein, and hypoallergenic pea-protein; and polysaccharides, such as cellulose, starch, and konjac, which have good gel-forming properties. Biopolymers derived from MOs, including microbial polysaccharides, probiotic bacteria, and YPs, can be certified as scalable and sustainable alternatives for CM bioengineering. The colloidal method combines the strengths of various structuring techniques, including electrospinning, microengraving, 3D bioprinting, microfluidic printing, and freezing casting, which have been extensively explored for biomedical scaffolding materials. Recent efforts have led to increased achievements and innovations in the context of these nature-derived biomaterials. However, there are still many challenges to overcome in the scaled bioengineering of CM. The collective knowledge that underlies the intersections of food fabrication and biomechanics will be crucial in achieving the aim of producing complex, whole-cut CM products.

CRediT authorship contribution statement

Pan Zhang: Formal analysis, Investigation, Writing – original draft, Funding acquisition. **Xu Zhao:** Methodology, Writing – review & editing. **Shiling Zhang:** Supervision, Validation. **Guoliang Li:** Methodology, Validation. **Adam C. Midgley:** Software, Methodology. **Yapeng Fang:** Conceptualization, Writing – review & editing. **Mouming Zhao:** Methodology, Validation. **Katsuyoshi Nishinari:** Conceptualization, Validation. **Xiaolin Yao:** Conceptualization, Writing – review & editing.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

No data was used for the research described in the article.

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References

Ahmad, K., Lim, J.H., 2021. Extracellular matrix and the production of cultured meat. Foods 10 (12), 3116.
Ahmad, K., Lim, J.-H., Lee, E.-J., Chun, H.-J., Ali, S., Ahmad, S.S., Shaikh, S., Choi, I., 2021. Extracellular matrix and the production of cultured meat. Foods 10 (12), 3116.
Ahmadi, R., Mordan, N., Forbes, A., Day, R.M., 2011. Enhanced attachment, growth and migration of smooth muscle cells on microcarriers produced using thermally induced phase separation. Acta Biomater. 7 (4), 1542–1549.
Andreassen, R.C., Rønning, S.B., Solberg, N.T., Grønlien, K.G., Kristoffersen, K.A., Høst, V., Kolset, S.O., Pedersen, M.E., 2022. Production of food-grade microcarriers based on by-products from the food industry to facilitate the expansion of bovine skeletal muscle satellite cells for cultured meat production. Biomaterials 286, 121602.
Andreeva, E., Matveeva, D., Zhidkova, O., Zhivodernikov, I., Kotov, O., Buravkova, L., 2022. Real and simulated microgravity: focus on mammalian extracellular matrix, 12 (9).
Argentati, C., Morena, F., Tortorella, I., Bazzucchi, M., Porcellati, S., Emiliani, C., Martino, S., 2019. Insight into mechanobiology: how stem cells feel mechanical forces and orchestrate biological functions. Int. J. Mol. Sci. 20 (21).
Ben-Arye, T., Shandalov, Y., Ben-Shaul, S., Landau, S., Zagury, Y., Ianovici, I., Lavon, N., Levenberg, S., 2020. Textured soy protein scaffolds enable the generation of three-dimensional bovine skeletal muscle tissue for cell-based meat. Nature Food 1 (4), 210–220.
Benjaminson, M.A., Gilchriest, J.A., Lorenz, M., 2002. In vitro edible muscle protein production system (MPPS): stage 1, fish. Acta Astronaut. 51 (12), 879–889.
Block, J., Schroeder, V., Pawelzyk, P., Willenbacher, N., Köster, S., 2015. Physical properties of cytoplasmic intermediate filaments. Biochim. Biophys. Acta 1853 (11 Pt B), 3053–3064.
Bodiou, V., Moutsatsou, P., Post, M.J., 2020. Microcarriers for upscaling cultured meat production. Front. Nutr. 7, 10.

Bolívar-Monsalve, E.J., Ceballos-González, C.F., 2022. One-step bioprinting of multi-channel hydrogel filaments using chaotic advection: fabrication of pre-vascularized muscle-like tissues, 11 (24), e2200448.
Bomkamp, C., Skaalure, S.C., 2022. Scaffolding biomaterials for 3D cultivated meat: prospects and challenges. Adv. Sci. 9 (3), e2102908.
Bomkamp, C., Skaalure, S.C., Fernando, G.F., Ben-Arye, T., Swartz, E.W., Specht, E.A., 2022. Scaffolding biomaterials for 3D cultivated meat: prospects and challenges. Adv. Sci. 9 (3), e2102908.
Boonen, K.J., Rosaria-Chak, K.Y., Baaijens, F.P., van der Schaft, D.W., Post, M.J., 2009. Essential environmental cues from the satellite cell niche: optimizing proliferation and differentiation. Am. J. Physiol. Cell Physiol. 296 (6), C1338–C1345.
Broucke, K., Van Pamel, E., Van Coillie, E., Herman, L., Van Royen, G., 2023. Cultured meat and challenges ahead: a review on nutritional, technofunctional and sensorial properties, safety and legislation. Meat Sci. 195, 109006.
Browe, D., Freeman, J., 2019. Optimizing C2C12 myoblast differentiation using polycaprolactone-polypyrrole copolymer scaffolds. J. Biomed. Mater. Res. 107 (1), 220–231.
Bruyère, C., Versaevel, M., Mohammed, D., Alaimo, L., Luciano, M., Vercruysse, E., Gabriele, S., 2019. Actomyosin contractility scales with myoblast elongation and enhances differentiation through YAP nuclear export. Sci. Rep. 9 (1), 15565.
Capri, M., Conte, M., Ciurca, E., Pirazzini, C., Garagnani, P., Santoro, A., Longo, F., Salvioli, S., Lau, P., Moeller, R., Jordan, J., Illig, T., Villanueva, M.M., Gruber, M., Bürkle, A., Franceschi, C., Rittweger, J., 2023. Long-term human spaceflight and inflammaging: does it promote aging? Ageing Res. Rev. 87, 101909.
Carnes, M.E., Pins, G.D., 2020. Etching anisotropic surface topography onto fibrin microthread scaffolds for guiding myoblast alignment. J. Biomed. Mater. Res. B Appl. Biomater. 108 (5), 2308–2319.
Chakraborty, M., Sivan, A., Biswas, A., Sinha, B., 2022. Early tension regulation coupled to surface myomerger is necessary for the primary fusion of C2C12 myoblasts. Front. Physiol. 13.
Chen, X., 2020. Uniaxial stretching of cell-laden microfibers for promoting C2C12 myoblasts alignment and myofibers formation, 12 (2), 2162–2170.
Chen, Y., Shafiq, M., Liu, M., Morsi, Y., Mo, X., 2020. Advanced fabrication for electrospun three-dimensional nanofiber aerogels and scaffolds. Bioact. Mater. 5 (4), 963–979.
Cheng, Y.W., Shiwarski, D.J., Ball, R.L., Whitehead, K.A., 2020. Engineering aligned skeletal muscle tissue using decellularized plant-derived scaffolds. ACS Biomater. Sci. Eng. 6 (5), 3046–3054.
Contessi Negrini, N., Toffoletto, N., Farè, S., Altomare, L., 2020. Plant tissues as 3D natural scaffolds for adipose, bone and tendon tissue regeneration. Front. Bioeng. Biotechnol. 8, 723.
Dell Orso, S., Juan, A.H., Ko, K.D., Naz, F., Perovanovic, J., Gutierrez-Cruz, G., Feng, X., Sartorelli, V., 2019. Single cell analysis of adult mouse skeletal muscle stem cells in homeostatic and regenerative conditions. Development (Cambridge, England) 146 (12).
Derakhti, S., Safiabadi-Tali, S.H., Amoabediny, G., Sheikhpour, M., 2019. Attachment and detachment strategies in microcarrier-based cell culture technology: a comprehensive review, Materials science & engineering. C, Materials for biological applications 103, 109782.
Ding, X., Zhuge, W., Zhang, Y., Ding, S., Wang, J., Zhou, G., 2023. Microfluidic generation of bioinspired core–shell structured microfibers for cultured meat. Chem. Eng. J. 478, 147467.
Distler, T., Solisito, A.A., Schneidereit, D., Friedrich, O., Detsch, R., Boccaccini, A.R., 2020. 3D printed oxidized alginate-gelatin bioink provides guidance for C2C12 muscle precursor cell orientation and differentiation via shear stress during bioprinting. Biofabrication 12 (4), 045005.
Dos Santos, A.E.A., Cotta, T., Santos, J.P.F., Camargos, J.S.F., do Carmo, A.C.C., Alcântara, E.G.A., Fleck, C., Copola, A.G.L., Nogueira, J.M., Silva, G.A.B., Andrade, L.O., Ferreira, R.V., Jorge, E.C., 2023. Bioactive cellulose acetate nanofiber loaded with annatto support skeletal muscle cell attachment and proliferation. Front. Bioeng. Biotechnol. 11, 1116917.
Dulgar-Tulloch, A.J., Bizios, R., Siegel, R.W., 2009. Human mesenchymal stem cell adhesion and proliferation in response to ceramic chemistry and nanoscale topography. J. Biomed. Mater. Res. 90 (2), 586–594.
Dumont, N.A., Bentzinger, C.F., Sincennes, M.C., Rudnicki, M.A., 2015. Satellite cells and skeletal muscle regeneration. Compr. Physiol. 5 (3), 1027–1059.
Dutta, S.D., Ganguly, K., Jeong, M.S., Patel, D.K., Patil, T.V., Cho, S.J., Lim, K.T., 2022. Bioengineered lab-grown meat-like constructs through 3D bioprinting of antioxidative protein hydrolysates, 14 (30), 34513–34526.
Enrione, J., Blaker, J.J., Brown, D.I., Weinstein-Oppenheimer, C.R., Pepczynska, M., Olguín, Y., Sánchez, E., Acevedo, C.A., 2017. Edible scaffolds based on non-mammalian biopolymers for myoblast growth. Materials 10 (12).
Ergene, E., Sezlev Bilecen, D., Kaya, B., Yilgor Huri, P., Hasirci, V., 2020. 3D cellular alignment and biomimetic mechanical stimulation enhance human adipose-derived stem cell myogenesis. Biomedical materials (Bristol, England) 15 (5), 055017.
Evertz, L.Q., Greising, S.M., Morrow, D.A., Sieck, G.C., Kaufman, K.R., 2016. Analysis of fluid movement in skeletal muscle using fluorescent microspheres. Muscle Nerve 54 (3), 444–450.
Fish, K.D., Rubio, N.R., Stout, A.J., Yuen, J.S.K., Kaplan, D.L., 2020. Prospects and challenges for cell-cultured fat as a novel food ingredient. Trends Food Sci. Technol. 98, 53–67.
Fontana, G., Gershlak, J., Adamski, M., Lee, J.S., Matsumoto, S., Le, H.D., Binder, B., Wirth, J., Gaudette, G., Murphy, W.L., 2017. Biofunctionalized plants as diverse biomaterials for human cell culture. Adv. Healthcare Mater. 6 (8).
Forcina, L., Miano, C., Pelosi, L., Musarò, A., 2019. An overview about the biology of skeletal muscle satellite cells. Curr. Genom. 20 (1), 24–37.
Furuhashi, M., Morimoto, Y., 2021a. Formation of contractile 3D bovine muscle tissue for construction of millimetre-thick cultured steak. NPJ science of food 5 (1), 6.
Furuhashi, M., Morimoto, Y., 2021b. Formation of contractile 3D bovine muscle tissue for construction of millimetre-thick cultured steak, 5 (1), 6.
Gilbert, P.M., Havenstrite, K.L., Magnusson, K.E., Sacco, A., Leonardi, N.A., Kraft, P., Nguyen, N.K., Thrun, S., Lutolf, M.P., Blau, H.M., 2010. Substrate elasticity regulates skeletal muscle stem cell self-renewal in culture. Science (New York, N.Y.) 329 (5995), 1078–1081.
Gillies, A.R., Lieber, R.L., 2011. Structure and function of the skeletal muscle extracellular matrix, Muscle & nerve, 44 (3), 318–331.
Gilpin, A., Yang, Y., 2017. Decellularization Strategies for Regenerative Medicine: from Processing Techniques to Applications, vol. 2017, 9831534.
Giordani, L., He, G.J., Negroni, E., Sakai, H., Law, J.Y.C., Siu, M.M., Wan, R., Corneau, A., Tajbakhsh, S., Cheung, T.H., Le Grand, F., 2019. High-dimensional single-cell cartography reveals novel skeletal muscle-resident cell populations. Mol. Cell 74 (3), 609–621.e6.
Gomez Romero, S., Boyle, N., 2023. Systems biology and metabolic modeling for cultivated meat: a promising approach for cell culture media optimization and cost reduction, 22 (4), 3422–3443.
Guan, X., Zhou, J., Du, G., Chen, J., 2022. Bioprocessing technology of muscle stem cells: implications for cultured meat. Trends Biotechnol. 40 (6), 721–734.
Guo, Y., Gilbert-Honick, J., Somers, S.M., Mao, H.Q., Grayson, W.L., 2019. Modified cell-electrospinning for 3D myogenesis of C2C12s in aligned fibrin microfiber bundles. Biochem. Biophys. Res. Commun. 516 (2), 558–564.
Haroon, M., Klein-Nulend, J., Bakker, A.D., Jin, J., Seddiqi, H., Offringa, C., de Wit, G.M. J., Le Grand, F., Giordani, L., Liu, K.J., Knight, R.D., Jaspers, R.T., 2021. Myofiber stretch induces tensile and shear deformation of muscle stem cells in their native niche. Biophys. J. 120 (13), 2665–2678.
Haroon, M., Bloks, N.G.C., Deldicque, L., Koppo, K., Seddiqi, H., Bakker, A.D., Klein-Nulend, J., Jaspers, R.T., 2022. Fluid shear stress-induced mechanotransduction in myoblasts: does it depend on the glycocalyx? Exp. Cell Res. 417 (1), 113204.
Holmes, J.T., Jaberansari, Z., Collins, W., Latour, M.L., Modulevsky, D.J., Pelling, A.E., 2022. Homemade bread: repurposing an ancient technology for in vitro tissue engineering. Biomaterials 280, 121267.
Ianovici, I., Zagury, Y., Redenski, I., Lavon, N., Levenberg, S., 2022. 3D-printable plant protein-enriched scaffolds for cultivated meat development. Biomaterials 284, 121487.
Ingber, D.E., Tensegrity, I., 2003. Cell structure and hierarchical systems biology. J. Cell Sci. 116 (Pt 7), 1157–1173.
Ippolito, A., Deshpande, V.S., 2023. Contact guidance via heterogeneity of substrate elasticity. Acta Biomater. 163, 158–169.
Iyer, S.R., Folker, E.S., Lovering, R.M., 2021. The nucleoskeleton: crossroad of mechanotransduction in skeletal muscle. Front. Physiol. 12, 724010.
Jabre, S., Hleihel, W., Coirault, C., 2021. Nuclear mechanotransduction in skeletal muscle. Cells 10 (2).
Jahangirian, H., Azizi, S., Rafiee-Moghaddam, R., Baratvand, B., Webster, T.J., 2019. Status of plant protein-based green scaffolds for regenerative medicine applications. Biomolecules 9 (10).
Jang, M., Scheffold, J., Røst, L.M., Cheon, H., Bruheim, P., 2022. Serum-free cultures of C2C12 cells show different muscle phenotypes which can be estimated by metabolic profiling. Sci. Rep. 12 (1), 827.
Jaques, A., Sánchez, E., Orellana, N., Enrione, J., Acevedo, C.A., 2021. Modelling the growth of in-vitro meat on microstructured edible films. J. Food Eng. 307, 110662.
Jeong, D., Seo, J.W., Lee, H.G., Jung, W.K., Park, Y.H., Bae, H., 2022. Efficient myogenic/adipogenic transdifferentiation of bovine fibroblasts in a 3D bioprinting system for steak-type cultured meat production, 9 (31), e2202877.
Jiao, A., Moerk, C.T., 2018. Regulation of skeletal myotube formation and alignment by nanotopographically controlled cell-secreted extracellular. Matrix 106 (6), 1543–1551.
Jiao, Z., Fu, C., Li, L., Wang, Z., Wang, Y., Shi, X., Zhang, P., 2019. Microcarriers with controllable size via electrified liquid jets and phase separation technique. Promote Cell Proliferation and Osteogenic Differentiation 2 (10), 4134–4141.
Jones, C.L., Penney, B.T., Theodossiou, S.K., 2023. Engineering cell-ECM-material interactions for musculoskeletal regeneration. Bioengineering (Basel, Switzerland) 10 (4).
Kim, B.S., Alcantara Jr., A.V., 2023. Comparative analysis of muscle atrophy during spaceflight. Nutritional Deficiency and Disuse in the Nematode Caenorhabditis elegans 24 (16).
Kim, W., Lee, H., Lee, J., Atala, A., Yoo, J.J., Lee, S.J., Kim, G.H., 2020. Efficient myotube formation in 3D bioprinted tissue construct by biochemical and topographical cues. Biomaterials 230, 119632.
Kolkmann, A.M., Post, M.J., 2020. Serum-free media for the growth of primary bovine myoblasts. Cytotechnology 72 (1), 111–120.
Kolodkin-Gal, I., Dash, O., Rak, R., 2023. Probiotic cultivated meat: bacterial-based scaffolds and products to improve cultivated meat. Trends Biotechnol.
Lee, K., Jackson, A., 2023. Bovine fibroblast-derived extracellular matrix promotes the growth and preserves the stemness of bovine stromal cells during in vitro expansion, 14 (4).
Lee, K.Y., Loh, H.X., Wan, A.C.A., 2021. Systems for muscle cell differentiation: from bioengineering to future food. Micromachines 13 (1).
Lepetit, J., 2008. Collagen contribution to meat toughness: theoretical aspects. Meat Sci. 80 (4), 960–967.
Levi, S., Yen, F.-C., Baruch, L., Machluf, M., 2022. Scaffolding technologies for the engineering of cultured meat: towards a safe, sustainable, and scalable production. Trends Food Sci. Technol. 126, 13–25.

Levy-Mishali, M., Zoldan, J., Levenberg, S., 2009. Effect of scaffold stiffness on myoblast differentiation, Tissue engineering. Part. Accel. 15 (4), 935–944.
Li, X., Chen, Z., Zhang, H., Zhuang, Y., Shen, H., Chen, Y., Zhao, Y., Chen, B., Xiao, Z., Dai, J., 2019. Aligned scaffolds with biomolecular gradients for regenerative medicine. Polymers 11 (2).
Li, Y., Liu, W., Li, S., Zhang, M., Yang, F., Wang, S., 2021. Porcine skeletal muscle tissue fabrication for cultured meat production using three-dimensional bioprinting technology. Journal of Future Foods 1 (1), 88–97.
Lindroos, B., Boucher, S., Chase, L., Kuokkanen, H., Huhtala, H., Haataja, R., Vemuri, M., Suuronen, R., Miettinen, S., 2009. Serum-free, xeno-free culture media maintain the proliferation rate and multipotentiality of adipose stem cells in vitro. Cytotherapy 11 (7), 958–972.
Listrat, A., Lebret, B., Louveau, I., Astruc, T., Bonnet, M., Lefaucheur, L., Picard, B., Bugeon, J., 2016a. How muscle structure and composition influence meat and flesh quality. Sci. World J. 2016, 3182746.
Listrat, A., Lebret, B., Louveau, I., Astruc, T., Bonnet, M., Lefaucheur, L., Picard, B., Bugeon, J., 2016b. How muscle structure and composition influence meat and flesh quality. TheScientificWorldJOURNAL 2016, 3182746.
Liu, Y., Wang, R., Ding, S., Deng, L., Zhang, Y., Li, J., Shi, Z., Wu, Z., Liang, K., Yan, X., Liu, W., Du, Y., 2022. Engineered meatballs via scalable skeletal muscle cell expansion and modular micro-tissue assembly using porous gelatin micro-carriers. Biomaterials 287, 121615.
Loreti, M., Sacco, A., 2022. The jam session between muscle stem cells and the extracellular matrix in the tissue microenvironment, 7 (1), 16.
Lu, H., Ying, K., Shi, Y., Liu, D., Chen, Q., 2022a. Bioprocessing by decellularized scaffold biomaterials in cultured meat: a review. Bioengineering (Basel) 9 (12).
Lu, H., Ying, K., Shi, Y., 2022b. Bioprocessing by decellularized scaffold biomaterials in cultured meat. A Review 9 (12).
Mackey, A.L., Magnan, M., Chazaud, B., 2017. Human skeletal muscle fibroblasts stimulate in vitro myogenesis and in vivo muscle regeneration, 595 (15), 5115–5127.
MacQueen, L.A., Alver, C.G., Chantre, C.O., Ahn, S., Cera, L., Gonzalez, G.M., O'Connor, B.B., Drennan, D.J., Peters, M.M., Motta, S.E., Zimmerman, J.F., Parker, K.K., 2019. Muscle tissue engineering in fibrous gelatin: implications for meat analogs. NPJ Sci Food 3, 20–31.
Madl, C.M., Flaig, I.A., Holbrook, C.A., Wang, Y.X., Blau, H.M., 2021. Biophysical matrix cues from the regenerating niche direct muscle stem cell fate in engineered microenvironments. Biomaterials 275, 120973.
Manzoor, M., Singh, J., Bandral, J.D., Gani, A., Shams, R., 2020. Food hydrocolloids: functional, nutraceutical and novel applications for delivery of bioactive compounds. Int. J. Biol. Macromol. 165, 554–567.
Modulevsky, D.J., Lefebvre, C., Haase, K., Al-Rekabi, Z., Pelling, A.E., 2014. Apple derived cellulose scaffolds for 3D mammalian cell culture. PLoS One 9 (5), e97835.
Nagamine, K., Sato, H., Kai, H., Kaji, H., Kanzaki, M., Nishizawa, M., 2018. Contractile skeletal muscle cells cultured with a conducting soft wire for effective, selective stimulation. Sci. Rep. 8 (1), 2253.
Narayanan, N., Jiang, C., Wang, C., Uzunalli, G., Whittern, N., Chen, D., Jones, O.G., Kuang, S., Deng, M., 2020a. Harnessing fiber diameter-dependent effects of myoblasts toward biomimetic scaffold-based skeletal muscle regeneration. Front. Bioeng. Biotechnol. 8, 203.
Narayanan, K.B., Zo, S.M., Han, S.S., 2020b. Novel biomimetic chitin-glucan polysaccharide nano/microfibrous fungal-scaffolds for tissue engineering applications. Int. J. Biol. Macromol. 149, 724–731.
Ng, S., Kurisawa, M., 2021. Integrating biomaterials and food biopolymers for cultured meat production. Acta Biomater. 124, 108–129.
Nishimura, T., 2015. Role of extracellular matrix in development of skeletal muscle and postmortem aging of meat. Meat Sci. 109, 48–55.
Norris, S.C.P., Kawecki, N.S., Davis, A.R., Chen, K.K., Rowat, A.C., 2022. Emulsion-templated microparticles with tunable stiffness and topology: applications as edible microcarriers for cultured meat. Biomaterials 287, 121669.
Orellana, N., Sánchez, E., Benavente, D., Prieto, P., Enrione, J., Acevedo, C.A., 2020a. A new edible film to produce in vitro meat. Foods 9 (2), 185.
Orellana, N., Sánchez, E., Benavente, D., Prieto, P., Enrione, J., 2020b. A new edible film to produce in vitro meat, 9 (2).
Pajčin, I., Knežić, T., Savic Azoulay, I., Vlajkov, V., Djisalov, M., Janjušević, L., Grahovac, J., Gadjanski, I., 2022. Bioengineering outlook on cultivated meat production. Micromachines 13 (3), 402.
Park, S., Jung, S., Heo, J., Koh, W.G., 2021. Chitosan/cellulose-based porous nanofilm delivering C-phycocyanin: a novel platform for the production of cost-effective cultured meat, 13 (27), 32193–32204.
Pegoraro, A.F., Janmey, P., Weitz, D.A., 2017. Mechanical properties of the cytoskeleton and cells. Cold Spring Harbor Perspect. Biol. 9 (11).
Petrany, M.J., Swoboda, C.O., Sun, C., 2020. Single-nucleus RNA-seq identifies transcriptional heterogeneity in multinucleated skeletal myofibers, 11 (1), 6374.
Pirsa, S., Hafezi, K., 2023. Hydrocolloids: structure, preparation method, and application in food industry. Food Chem. 399, 133967.
Prüller, J., Mannhardt, I., Eschenhagen, T., Zammit, P.S., 2018. Satellite cells delivered in their niche efficiently generate functional myotubes in three-dimensional cell culture, 13 (9), e0202574.
Rao, K.M., Kim, H.J., Won, S., Choi, S.M., Han, S.S., 2023. Effect of grape seed extract on gelatin-based edible 3D-hydrogels for cultured meat application. Gels (Basel, Switzerland) 9 (1), 65.
Reiss, J., Robertson, S., Suzuki, M., 2021. Cell sources for cultivated meat: applications and considerations throughout the. Production Workflow 22 (14).
Rojas-Rodríguez, M., Fiaschi, T., Mannelli, M., Mortati, L., Celegato, F., Wiersma, D.S., 2023. Cellular contact guidance on liquid crystalline networks with anisotropic roughness, 15 (11), 14122–14130.
Salehi, A., AminMohammadi, Mohammad, JavadShahsavarani, HoseinShokrgozar, AliAlipour, Mohammad, Atefeh, 2021. Natural cellulose-based scaffold for improvement of stem cell osteogenic differentiation. J. Drug Deliv. Sci. Technol. 63 (1).
Seah, J.S.H., Singh, S., Tan, L.P., 2022a. Scaffolds for the manufacture of cultured meat. Crit. Rev. Biotechnol. 42 (2), 311–323.
Seah, J.S.H., Singh, S., Tan, L.P., 2022b. Scaffolds for the manufacture of cultured meat, 42 (2), 311–323.
Seah, J.S.H., Singh, S., Tan, L.P., Choudhury, D., 2022c. Scaffolds for the manufacture of cultured meat. Crit. Rev. Biotechnol. 42 (2), 311–323.
Shahin-Shamsabadi, A., Selvaganapathy, P.R., 2022. Engineering murine adipocytes and skeletal muscle cells in meat-like constructs using self-assembled layer-by-layer biofabrication: a platform for development of cultivated meat. Cells Tissues Organs 211 (3), 304–312.
Shi, G., Zhang, P., Zhang, X., Li, J., Zheng, X., Yan, J., Zhang, N., Yang, H., 2023. The spatiotemporal heterogeneity of the biophysical microenvironment during hematopoietic stem cell development: from embryo to adult. Stem Cell Res. Ther. 14 (1), 251.
Singh, A., Kumar, V., Singh, S.K., Gupta, J., Kumar, M., Sarma, D.K., Verma, V., 2023a. Recent advances in bioengineered scaffold for in vitro meat production, 391 (2), 235–247.
Singh, A., Kumar, V., Singh, S.K., Gupta, J., Kumar, M., Sarma, D.K., Verma, V., 2023b. Recent advances in bioengineered scaffold for in vitro meat production. Cell Tissue Res. 391 (2), 235–247.
Skillin, N.P., Kirkpatrick, B.E., Herbert, K.M., Nelson, B.R., Hach, G.K., Günay, K.A., Khan, R.M., DelRio, F.W., White, T.J., Anseth, K.S., 2023. Stiffness anisotropy coordinates supracellular contractility driving long-range myotube-ECM alignment. bioRxiv.
Su, L., Jing, L., 2023. 3D-Printed prolamin scaffolds for cell-based meat culture, 35 (2), e2207397.
Su, L., Jing, L., Zeng, X., Chen, T., Liu, H., Kong, Y., Wang, X., Yang, X., Fu, C., Sun, J., Huang, D., 2023. 3D-Printed prolamin scaffolds for cell-based meat culture. Adv. Mater. 35 (2), e2207397, 2207405.
Takahashi, H., Nakamura, A., Shimizu, T., 2021. Simulated microgravity accelerates aging of human skeletal muscle myoblasts at the single cell level. Biochem. Biophys. Res. Commun. 578, 115–121.
Tao, J., Choudhury, M.I., Maity, D., Kim, T., Sun, S.X., Fan, C.M., 2023. Mechanical compression creates a quiescent muscle stem cell niche. Commun. Biol. 6 (1), 43.
Tarantino, U., Cariati, I., Marini, M., D'Arcangelo, G., Tancredi, V., Primavera, M., Iundusi, R., Gasbarra, E., Scimeca, M., 2020. Effects of simulated microgravity on muscle stem cells activity. Cell. Physiol. Biochem. : international journal of experimental cellular physiology, biochemistry, and pharmacology 54 (4), 736–747.
Thorrez, L., DiSano, K., Shansky, J., Vandenburgh, H., 2018. Engineering of human skeletal muscle with an autologous deposited extracellular matrix. Front. Physiol. 9, 1076.
Uehara, T.M., Paino, I.M.M., Santos, F.A., Scagion, V.P., Correa, D.S., Zucolotto, V., 2020. Fabrication of random and aligned electrospun nanofibers containing graphene oxide for skeletal muscle cells scaffold. Polym. Adv. Technol. 31 (6), 1437–1443.
Uroz, M., Wistorf, S., Serra-Picamal, X., Conte, V., 2018. Regulation of cell cycle progression by cell-cell and cell-matrix forces, 20 (6), 646–654.
van Santen, V.J.B., Klein-Nulend, J., Bakker, A.D., Jaspers, R.T., 2022. Stiff matrices enhance myoblast proliferation, reduce differentiation, and alter the response to fluid shear stress in vitro. Cell Biochem. Biophys. 80 (1), 161–170.
Verbruggen, S., Luining, D., van Essen, A., Post, M.J., 2018. Bovine myoblast cell production in a microcarriers-based system. Cytotechnology 70 (2), 503–512.
Vogt, N., 2021. Assembloids. Nat. Methods 18 (1), 27.
Wang, S., Reuveny, A., Volk, T., 2015. Nesprin provides elastic properties to muscle nuclei by cooperating with spectraplakin and EB1. J. Cell Biol. 209 (4), 529–538.
Wang, L., Li, Y., Huang, G., Zhang, X., Pingguan-Murphy, B., Gao, B., Lu, T.J., Xu, F., 2016. Hydrogel-based methods for engineering cellular microenvironment with spatiotemporal gradients. Crit. Rev. Biotechnol. 36 (3), 553–565.
Wang, Y., Zhong, Z., Wang, R., Munawar, N., Zan, L., Zhu, J., 2023. Effects of proanthocyanidins and dialdehyde chitosan on the proliferation and differentiation of bovine myoblast for cultured meat production. Int. J. Biol. Macromol. 246, 125618.
Wang, Y., Zhong, Z., Munawar, N., Zan, L., Zhu, J., 2024. 3D edible scaffolds with yeast protein: a novel alternative protein scaffold for the production of high-quality cell-cultured meat. Int. J. Biol. Macromol. 259, 129134.
Wei, Z., Dai, S., Huang, J., Hu, X., Ge, C., Zhang, X., 2023. Soy protein amyloid fibril scaffold for cultivated meat application, 15 (12), 15108–15119.
Wollschlaeger, J.O., Maatz, R., Albrecht, F.B., Klatt, A., Heine, S., 2022. Scaffolds for cultured meat on the basis of polysaccharide hydrogels enriched with plant-based proteins, 8 (2).
Xiang, N., Yuen Jr., J.S.K., Stout, A.J., Rubio, N.R., Chen, Y., Kaplan, D.L., 2022a. 3D porous scaffolds from wheat glutenin for cultured meat applications. Biomaterials 285, 121543.
Xiang, N., Yao, Y., Yuen Jr., J.S.K., Stout, A.J., Fennelly, C., Sylvia, R., Schnitzler, A., Wong, S., Kaplan, D.L., 2022b. Edible films for cultivated meat production. Biomaterials 287, 121659.
Xu, T., Miszuk, J.M., Zhao, Y., Sun, H., Fong, H., 2015. Electrospun polycaprolactone 3D nanofibrous scaffold with interconnected and hierarchically structured pores for bone tissue engineering. Adv. Healthcare Mater. 4 (15), 2238–2246.

Yan, Q., Fei, Z., Li, M., Zhou, J., 2022. Naringenin promotes myotube formation and maturation for cultured meat production, 11 (23).
Yang, L., Yaseen, M., Zhao, X., Coffey, P., Pan, F., Wang, Y., Xu, H., Webster, J., Lu, J.R., 2015. Gelatin modified ultrathin silk fibroin films for enhanced proliferation of cells. Biomed. Mater. 10 (2), 025003.
Yap, W.S., Choudhury, D., Suntornnond, R., 2023. Towards biomanufacturing of cultured meat. Trends Biotechnol. 41 (3), 292–294.
Yen, F.C., Glusac, J., 2023. Cultured meat platform developed through the structuring of edible microcarrier-derived microtissues with oleogel-based fat substitute, 14 (1), 2942.
Yeo, M., Kim, G.H., 2018. Anisotropically Aligned Cell-Laden Nanofibrous Bundle Fabricated via Cell Electrospinning to Regenerate Skeletal Muscle Tissue, Small (Weinheim an der Bergstrasse. Germany) 14 (48), e1803491.
Zernov, A., Baruch, L., Machluf, M., 2022. Chitosan-collagen hydrogel microparticles as edible cell microcarriers for cultured meat. Food Hydrocolloids 129, 107632.
Zhang, L., Le Coz-Botrel, R., Beddoes, C., Sjöström, T., Su, B., 2017. Gelatin freeze casting of biomimetic titanium alloy with anisotropic and gradient pore structure. Biomedical materials (Bristol, England) 12 (1), 015014.
Zheng, Y.Y., Shi, Y.F., Zhu, H.Z., Ding, S.J., Zhou, G.H., 2022a. Quality evaluation of cultured meat with plant protein scaffold. Food Res. Int. 161, 111818.
Zheng, Y.Y., Chen, Y., Zhu, H.Z., Li, C.B., Song, W.J., Ding, S.J., Zhou, G.H., 2022b. Production of cultured meat by culturing porcine smooth muscle cells in vitro with food grade peanut wire-drawing protein scaffold. Food Res. Int. 159, 111561.
Zhu, D., Trinh, P., Li, J., Grant, G.A., Yang, F., 2021. Gradient hydrogels for screening stiffness effects on patient-derived glioblastoma xenograft cellfates in 3D. J. Biomed. Mater. Res. 109 (6), 1027–1035.