This SLAPS: Stereolithography (SLA) 3D Printing of Scaffolding for Cellular Agriculture made from Functionalized Chitin

Cultivate Tomorrow 2022 Hackathon Proposal

Abstract

Conventional meat production is severely unsustainable as it depletes valuable land and water resources while producing significant greenhouse gas emissions and enabling the mistreatment of animals in the process. The cellular agriculture (cell ag) industry has come about in the last decade to combat the increasing need for sustainable meat with new technologies involving growing cells in large efficient bioreactors. Unfortunately, the industry has thus far been plagued with a lack of inexpensive, abundant, environmentally friendly scaffolds on which to culture those cells in 3D environments, necessary for cell differentiation. Here, we propose utilizing a current waste stream in the seafood industry, chitin, along with laser-based photoinitiation 3D printing technology to produce chitin-based hydrogel scaffolds, underutilized resources, and technologies in the industry. This new technology will provide unrivaled 3D spatial control to create whole cuts of meat that are visually indistinguishable from conventional meat products. When combined with cells in cultured meat, these novel scaffolds save money, time, resources, and the environment in the long term.

Introduction

Humans started incorporating meat and marrow from large animals into their diets after an evolutionary change approximately 2.6 million years ago (Pobiner, 2013). Since then, meat consumption has become a significant part of the human diet. With advancements in agricultural technology during the past century, animal products have become easier to produce on a large scale, and the demand for meat has risen. Meat consumption has increased by 40 percent between 1961 and 2017 in the United States and 58 percent between 1998 and 2018 globally (Christen, 2021). The global meat consumption average per capita continues to increase due to various factors, including population growth and higher incomes (Godfray et al., 2018). Increased demands for meat have caused significant environmental strain as such food systems require more

energy, land, and water than plant-based diets (Pimentel and Pimentel, 2003). Additionally, animal agriculture has contributed significantly to global greenhouse gas emissions, driving climate change. According to the United Nations Food and Agriculture Organization, global livestock alone produces 7.1 Gigatonnes of CO_2 annually, equating to nearly 14.5 percent of all man-made greenhouse gas emissions (Food and Agriculture Organization, n.d.). On top of terrestrial meat production, modern seafood processing contributes to a massive waste problem, as the inedible components of seafood such as shells, skin, heads, and scales end up being discarded and not repurposed. Improper seafood waste disposal not only contributes to adverse environmental and human health impacts but also overlooks high-value biopolymers such as chitin and chitosan contained in these discarded products (Yadav, 2019). With the global population estimated to reach 10.9 billion by the end of the century (Roser, 2013), and the growing demand for animal products, the need for sustainable protein sources is dire. This proposal underlines the potential for chitin from seafood waste, combined with the emerging technology of cellular agriculture as a method of producing sustainable protein.

Cultured meat is a relatively new technology that utilizes the fundamentals of tissue engineering to culture animal stem cells as an alternative to typical meat sources. There is a developing interest in creating structurally complex cell-cultured products within cellular agriculture that appear identical to animal-based products. A heavily discussed approach to creating these complex, three-dimensional cell products is utilizing scaffolds seeded with cells. Scaffolds in cell and tissue culture are constructs that support and control the adhesion, assembly, differentiation, and growth. The constructs are designed to mimic the extracellular matrix (ECM) found in live animal tissue (See **Figure 1**). The ECM is a network of macromolecules that are organized to induce tissue-specific cell organization and differentiation (Yue, 2015). Scaffolds also provide a host environment for cells to grow and respond to their environment and sometimes contain growth factors that assist cell growth (Chan and Leong, 2008).

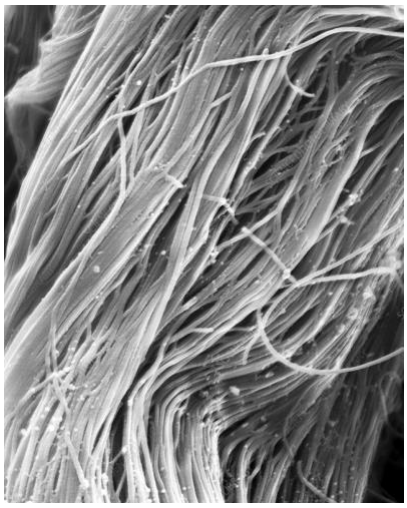


Figure 1: Example of an extracellular muscle matrix of collagen observed through a scanning electron micrograph. (*Kunkel, n.d.)*

Scaffolds for cell culture are typically composed of polymeric biomaterials, the material used ranges depending on the tissue culture of interest. Self-secreted ECM, collagen, gelatin, alginate, chitosan, and synthetic polymers, such as poly(*N*-isopropylacrylamide), are some materials used as scaffold material.

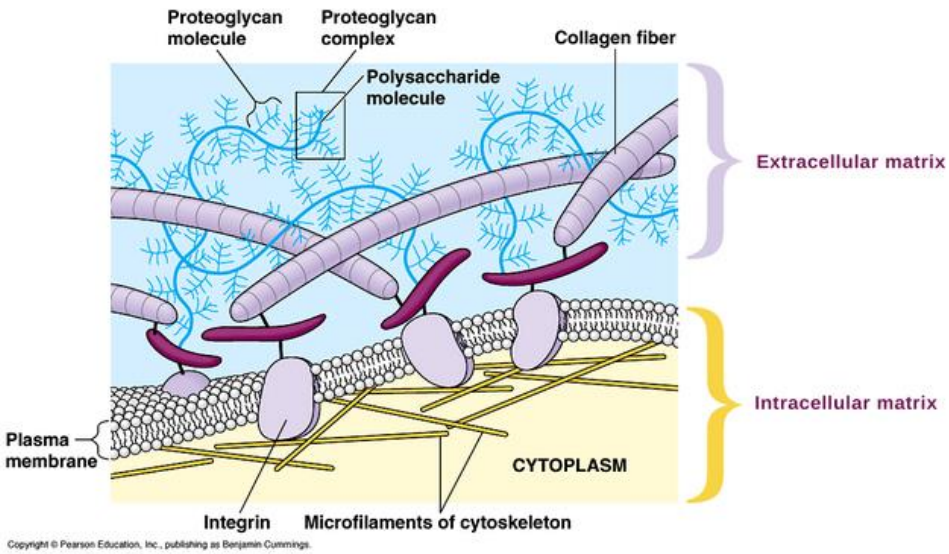


Figure 2: A sample of the interactions between the ECM and cells (*Pearson Education Inc.)*

Mimicking the complex composition of the ECM poses many challenges. To design a structure that assists in cell growth and differentiation similarly to the ECM, some high

priority features need to be taken into account, including construct architecture, cell compatibility, bioactivity, and edibility.

Architecture

The scaffold's three-dimensional architecture should be porous to mimic a vascular network to allow gas and nutrient exchange. The scaffold should be free-standing and robust enough to maintain shape and stability as the weight of the tissue mass increases during the growth process. Lastly, the structure should be degradable so that the cells can grow around and replace existing scaffolding structures.

Cell Compatibility and Bioactivity

In a functional scaffold, cells should be able to adhere to the material, undergo cell differentiation, and grow. Thus, scaffolds should be compatible with the cell type of interest. Scaffolds containing adhesion molecules and growth factors can help facilitate the speed and specificity of tissue growth (Chan and Leong, 2008).

Edibility

To date, most of the scaffold research has focused on scaffolding in biomedical applications (Young and Skrivergaard, 2020). In cultured meat, scaffold biocompatibility and edibility are integral for the application in food.

Printing Process

Additive manufacturing or three-dimensional (3D) printing has been a recent advancement in the food technology industry. The (3D) printing process deposits, joins or solidifies materials layer by layer to create precise 3D objects. The object is initially designed as a model on computer-aided design (CAD) software, where it is then transferred to a 3D printer to create the physical construct. Various additive manufacturing processes exist, and the two most common methods are extrusion and vat photopolymerization. Extrusion processes use a filament to deposit materials via an extruder layer-by-layer into three-dimensional objects. Variations of this method have been developed since the first extrusion-based process was created, making it suitable for a wide variety of material feedstocks. Light-based, vat photopolymerization printing processes such as stereolithography (SLA) utilize lasers and photocurable resins to selectively polymerize a liquid resin upon irradiation. While extrusion-based printing has

a broader range of materials compatible with its processes, vat photopolymerization methods are advantageous due to their ability to rapidly fabricate high resolution and more structurally complex objects.

The high resolution, structural specificity, and rapid manufacturing speeds make the SLA printing process an exciting candidate for use in tissue engineering, and to date, it has not been used to fabricate edible materials. However, to create a viable resin that can be used in cellular agriculture, many considerations must be taken into account, including the chemical and physical properties of the formulation, product safety, material availability, and production capacity (Jahagirian et al., 2019).

One of the primary challenges faced in formulating novel resins for SLA is optimizing the rheological properties of the resin based on concentration and molecular weight. A desirable viscosity is below 10 Pa·s in order for the resin to redistribute on the resin platform quickly and evenly between layers. Resin viscosity is commonly reduced by increasing printing temperatures or diluting the formula with additives. An alternative tactic for decreasing the viscosity of the resin is by altering the molecular architecture of the resin. Viscosity reduction is done by synthesizing branched, dendritic, or cross-linked polymers with lower intrinsic viscosity than their linear counterparts.

Utilizing SLA for tissue engineering applications has been limited in the past by the existence and availability of resins that are both biocompatible and polymerizable in the photopolymerization process. Recent advancements in biocompatible SLA resins include materials such as synthetic poly(alkyl fumarate) and poly(lactides), as well as functionalized proteins such as gelatin methacrylate and functionalized polysaccharides (Smith et al., 2020). Polysaccharides are of particular interest for this process because they are water-soluble and contain pendant groups that can be functionalized to tune the physical and chemical properties of the resin and the resultant printed construct. Functionalized polysaccharides including chitosan (Cisneros et al., 2021), alginate, cellulose (Gauss, 2021), and pullulan (Della Giustina, 2019) have proven to be printable resin matrices in various light-based 3D printing processes and have shown good cell adhesion properties in the cell culturing process (Tai et al., 2019). To date, most biomaterials research for tissue engineering focuses on applications in biomedicine, while scaffolds for cell-cultured products remain in their infancy. Medical device materials and

in vitro tissue culture standards differ from food-grade materials. Therefore, further research is required to design and use polymeric scaffolding for food products, with the most significant consideration being that the scaffold construction materials must be food grade and edible (Seah et al., 2021).

Analysis of Companies Currently in the Cellular Agriculture and Cultivated Meat Industry

There has been some ongoing research regarding 3D printing's applicability for food and the usage of chitin, and its deacetylated form, chitosan, to produce scaffolds for cellular agriculture. However, not many companies currently exist researching 3D printed chitin scaffolds.

Additive manufacturing technologies have been utilized to create structurally complex alternative meat products by MeaTech, Redefine Meat, and Aleph Farms. MeaTech is an Israeli company that applies extrusion-based 3D printing technology to cellular agriculture with unique bio-inks from specific cell lines and scaffolds in specific 3D bioprinters. The cells are incubated to promote cell growth and tissue formation. According to MeaTech, this method requires less time than conventional culturing methods. In December 2021, MeaTech produced the world's largest lab-grown steak (3.67 oz) after over a year of cultivated meat research using novel 3D printing technology (Romaine, 2021). Redefine Meat is another Israeli company working towards producing plant-based meat products using 3D printing technology. The company has released a series of plant-based 3D printed meat, including Redefine Kabab and sausages (Cottingham, 2021). Additionally, Aleph Farms, another Israeli startup, has been working on creating lab-grown meats since 2018, primarily relying on live animal cell cultures to create different types of meat. They recently began working on 3D bio printed meat, hoping that they could produce such meat in outer space without relying significantly on natural resources found on Earth (Cottingham, 2021). Such companies have been at the forefront of applying 3D printing technology when producing meat alternatives using primarily extrusion-based techniques.

Figure 3: Redefine Meat 3D's printed meat sample (*Reuters*). Older jet 3D printing technology.

Several companies have been experimenting with using chitin as a scaffold when creating alternative meats ut have not utilized 3D printing technologies to implement their solutions. For example, one company, Excell, is creating chitin-based scaffolds. Excell is currently using fungal mycelium as a source of chitin to create scaffolds for various applications. Excell currently cultivates lab-grown mycelium and subsequently chitin, as opposed to our proposed 3D printed chitin-based scaffolds.

Proposed Solution

We propose a cogent alternative to produce structurally complex cultured meat products by utilizing laser-based photoinitiation printing technology to assemble chitin-based hydrogel cell scaffolds. The photopolymerizable SLA resin comprises water-soluble modified α-chitin functionalized with an edible radically polymerizable monomer and a plant-based organic photoinitiator system. Printing using SLA has not been previously reported, to our knowledge, applied to the field of cell ag. SLA has the significant advantage of ultra-fine spatial control, unmatched by gen 1 extrusion-based 3D printers and bioprinters.

Chitin

Chitin is the second most abundant polysaccharide in nature. It has high promise for use in cultured food products due to its biodegradability, biocompatibility, and easily modifiable chemical structure (see **Figure 4**) (Pahlevanzadeh et al., 2020).

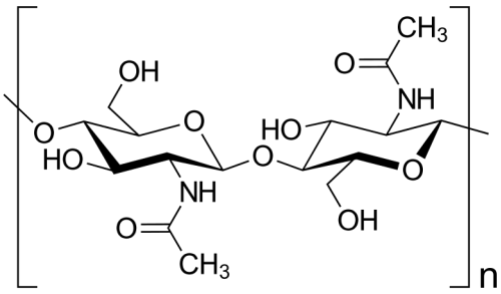


Figure 4: α-chitin molecular structure (*Wikipedia)*

In addition to its unique physical and chemical properties, chitin is abundant, naturally occurring, and low-cost to purchase and/or extract. Chitin can be derived from upcycled arthropod exoskeletons, an enormous waste product of the seafood industry, and algae and fungi (Kurita, 2001). Chitin has low water solubility in its natural form due to the N-acetylene groups. However, chitin dissolved in aqueous sodium hydroxide undergoes rapid N-deacetylation, increasing the water solubility of the molecule. Alternatively, naturally occurring polysaccharide chitosan, which is deacetylated chitin, can be used as an alternative starting material and functionalized to the appropriate ratios to allow water solubility. The free amine groups on the deacetylated chitin can be functionalized with a food-safe, radically polymerizable monomer, incorporated via a nucleophilic addition or substitution. This functionalization step will eventually allow for the photochemical cross-linking of chitin backbones by a food-grade polymer to form a hydrogel scaffold construct.

Commercial Chitin Reclamation from Seafood Waste

Industrially, chitin is produced via chemical extraction or microbial fermentation. According to "*Methods of Chitin Production a Short Review*" by Pighinelli et al., chemical extraction of chitin involves deproteinization and demineralization (2019). These steps are subsequently augmented with depigmentation and deodorization if necessary. An alkaline solution is used for deproteinization followed by strong acid for demineralization to yield chitin in a powdered form. Although the most widely used, the chemical extraction method utilized hazardous chemicals generating effluent that might harm the environment if not correctly disposed of.

Alternatively, research has shown that co-fermenting crustaceous waste with *Lactobacillus plantarum* subsp. *plantarum ATCC 14917* and *Bacillus subtilis* subsp. *subtilis ATCC 6051* and utilizing red grape pomace, an agricultural waste product, as a carbon source produces chitin with a high degree of deacetylation of 72.90%, comparable to chemically extracted chitin at a commercial scale (Tan et al., 2020).

Microbial fermentation mentioned above is a promising method for chitin reclamation since it utilizes agricultural waste and waste from the seafood industry while minimizing the usage of hazardous chemicals. However, for initial research and product development, the team plans to source chitin from commercial vendors with a vision to vertically integrate the supply post scaffold launch with a time horizon of 3-5 years in mind.

Deacetylation and characterization of chitin

Chemically extracted chitin or co-fermented chitin will be deacetylated by dissolving the dried powder in a so% aqueous sodium hydroxide solution. The reaction will be washed and purified to yield 70-90% deacetylated chitin. The degree of chitin deacetylation (DDA%) can be determined using an elemental analyzer to find the carbon to nitrogen (C/N) ratio (Ashraf et al., 2016). The C/N ratio using the following equation:

$$DDA\% = \left(1 - \frac{C/N - 5.145}{6.861 - 5.145}\right) \times 100$$

where the C/N ratio of completely deacetylated chitin (chitosan) is 5.145, and the C/N ratio of completely acetylated chitin is 6.861(Abdulkarim et al., 2013).

Functionalization and characterization of chitin with photopolymerizable monomer

A non-extensive list of monomer targets that have the chemical properties suitable for both functionalization (carbonyl functionality) and radical polymerization (conjugated alkenes) have been identified and displayed in **Figure 5.**

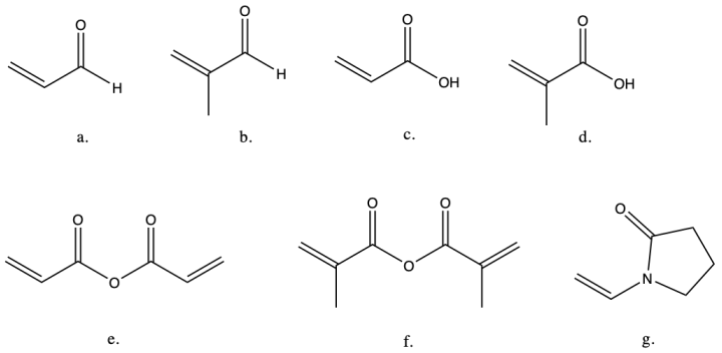


Figure 5. List of potential monomeric candidates for chitin functionalization: (a) propenal, (b) methylpropenal, (c) acrylic acid, (d) methacrylic acid, (e) acrylic anhydride, (f) methacrylic anhydride, and (g) vinyl pyrrolidone

The free amine groups will be modified by adding the selected monomer in a mildly acidic solution. The functionalized polymer will be extracted from the solution and purified in the aqueous phase using liquid-liquid extraction.

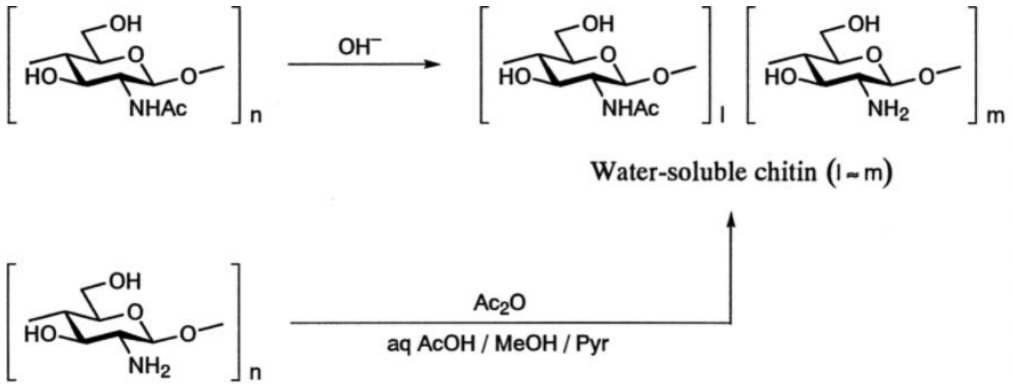


Figure 6: Deacetylation of chitin in a hydroxide solution and acetylation of chitosan to create water-soluble chitin (*Kurita, 2001*)

For optimal water solubility of chitin at neutral pH, the overall degree of deacetylation should be 0.45-0.55, so the final ratio of functionalized to free amine is expected to be approximately 50:50 but may vary and will be adjusted depending on the structure and solubility of the selected monomer. Functionalization of the polymer will be characterized via elemental analysis.

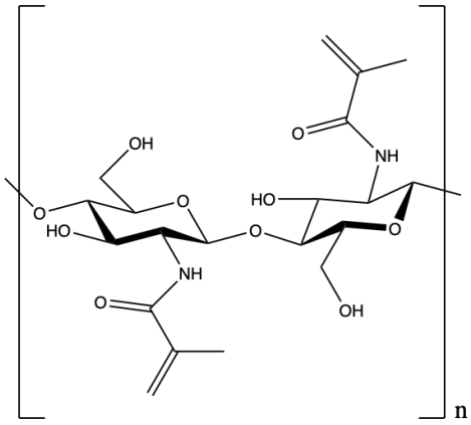


Figure 7: Methyl Methacrylate-Functionalized Chitin

Chitin undergoes hydrolysis under acidic conditions, so methacrylic acid and acrylic acid may pose challenges to the functionalization process if the degree of hydrolysis causes an undesirable degree of depolymerization of chitin. However, hydrolysis may not necessarily be counterproductive, as hydrolysis may help to optimize the rheological properties of the resultant resin, which will be discussed later in this section. Each monomer will be tested to determine the degrees and rates of functionalization, water solubility, and printability, and the resultant polymer will be screened for biocompatibility. The monomer and polymer that optimizes the desirable properties will be selected as the final candidate for the resin.

Hydrolysis of the chitin backbone will be assessed using static light scattering or gel permeation chromatography (GPC) to determine the average molecular weight of chitin before and after the functionalization process (Wineinger et al., 2020).

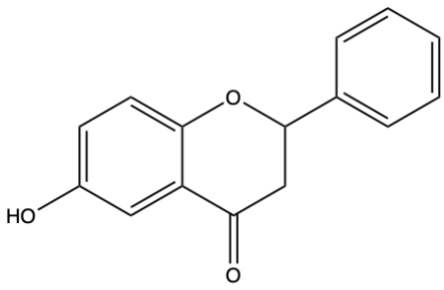
High throughput screening for selection of resin comonomer

The addition of a radically-polymerizable co-monomer induces cross-linking between the chitin backbones, leading to faster photocuring and thus better printability of the resin. In the last two decades, polymer arrays have become widely available (Tourniaire et al., 2006). A high throughput screen (HTS) will be run on a library of known food-safe monomers and polymers to determine cell compatibility and find a platform of likely candidates for the material. These arrays are glass slides coated with a non-cell-adhesive layer of polyHEMA, followed by spotting monomers and polymers of interest at

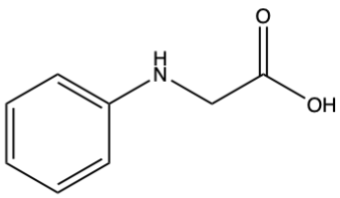
a density of 2,000-10,000 spots per slide. Cells of interest are then cultured on the slides to observe attachment and proliferation. After a period of 1-10 days cell culture, spots are observed visually under a microscope with or without DAPI staining. The spots having the most significant number of cells per spot materials will be chosen for comonomer.

Photoinitiator Selection

A photo initiating system induces the formation of radicals upon irradiation, which begins the material polymerization process. Most SLA printers use a 405 nm wavelength laser to irradiate the resin, and thus the photoinitiator must have high absorbance at 405 nm. Historically, photoinitiators have been made of heavy metal complexes and/or strong and toxic acids, resulting in materials from SLA that are not biocompatible. A growing interest in both sustainability and the use of SLA and other photopolymerization-based processes for biomaterials applications has spurred research into discovering and developing naturally derived, biocompatible photoinitiators and photoinitiating systems. For use in cultured cell food products, it is essential to have a non-toxic, edible photo initiating system. Various plant-derived photocatalysts and photocatalytic systems have been studied and developed in recent years, including systems based on coumarins, chalcones, and chromones (Giacoletto and Dumur, 2021). In one study, a two-component photoinitiating system composed of plant metabolite, 6-hydroxyflavone, and the amino acid derivative, N-phenylglycine showed successful free radical polymerization of acrylate and methacrylate monomers at 405 nm and were successfully applied in the fabrication of 3D printed photopolymers (Al Mousawi et al., 2020).



6-Hydroxyflavone



N-Phenylglycine

Figure 8. Proposed photocatalytic system composed of 6-Hydroxyflavone and N-Phenylglycine

Resin Formulation

The resin will be formulated by dissolving the selected comonomer (5 wt %) in water, followed by the slow addition of functionalized chitin (30 wt %), and the solution will be mixed until dissolved. Next, 6-hydroxy flavone (0.5 wt %) and N-phenyl glycine (1 wt%) will be added sequentially and dissolved in the resin.

Rheometry will be used to evaluate the viscosity of the pre-print resin to ensure that the intrinsic viscosity of the solution is minimized (under 10 Pa·s) for optimal printing. Adjustments to formulation will be made using viscometric constants based on the Mark-Houwink-Sakurada (MHS) equation with constants modified based on the degree of acetylation and ionic strength of the solution (Kasaai, 2007).

$$[1]\ [\eta] = KM_v^a$$

$$[2]\ a = 0.6202 + \frac{0.699x}{0.4806+x}$$

$$[3]\ x = \frac{DA}{pH \cdot \mu}$$

[1] Mark-Houwink-Sakurada equation where $[\eta]$ is the intrinsic viscosity of the resin, M_v is the viscosity-average molecular weight of, the polymer and a [2], [3] is a material-specific constant for chitosan based on the degree of acetylation (DA) and the pH and ionic strength (μ) of the solvent.

The intrinsic viscosity of the formula is primarily controlled by polymer concentration and molecular weight, with minor contributions from the degree of acetylation of chitin and the pH and ionic strength of the solution. The molecular weight and/or the concentration of the functionalized chitin may need to be modified to achieve printable resin viscosity. An acid-based degradation method can be used to induce hydrolysis of the polysaccharide's glycosidic linkages, decreasing the molecular weight and thus the viscosity of the formulation. Alternatively, creating a graft copolymer or hyperbranched architecture on the functionalized sites could also decrease the viscosity of the solution. If the viscosity of the resin needs to be increased, the concentration of chitin in the formulation can be increased.

The cure rate or the rate of light-induced crosslinking of the resin will be determined by photo rheometry to ensure efficient print times. The amount of 6-hydroxyflavone and N-phenylglycine in the formulation may need to be increased or decreased depending on the rate of photopolymerization calculated by the photo rheometer.

Fabrication of Chitin-based Hydrogels

Functionalized chitin hydrogels will be fabricated using a Formlabs Form 2 Printer. 3D constructs with high porosity for gas and nutrient diffusion will be designed using computer-aided design software Autodesk Fusion 360. Upon print completion, the printed constructs will be removed from the printer plate, rinsed with DI water to remove uncured resin, and post-cured in a photocuring chamber to induce further cross-linking.

Figure 9: Formlabs Form 2 printer (*TechCrunch*)

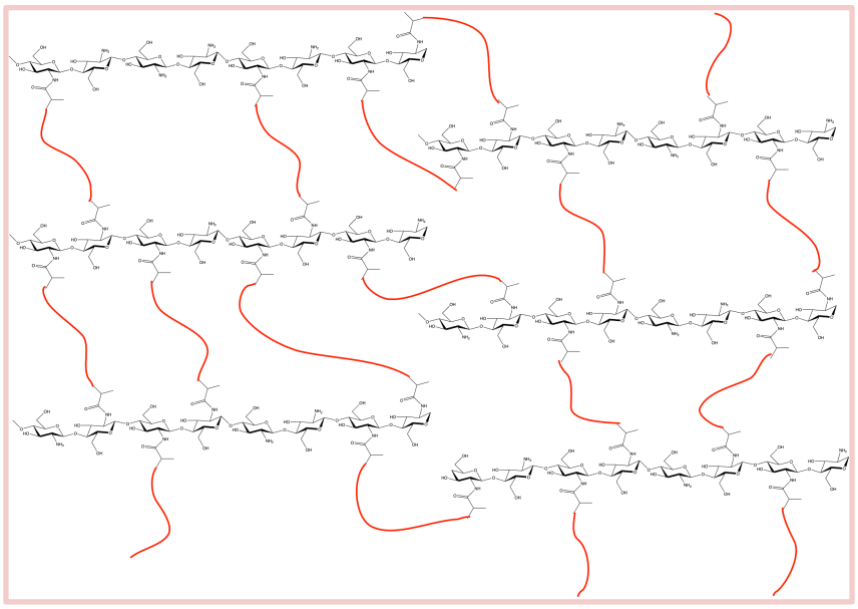


Figure 10: Molecular depiction of final network hydrogel: methacrylated chitin photochemically cross-linked by co-monomer (red)

Cell culture and biocompatibility assessments will be carried out using an HTS process. Chitin-based hydrogel samples will be inserted into poly(styrene) 96-well culture plate wells. Selected cell lines from a variety of species will be seeded onto the films at a density of 1×10^5 cells/cm^2 and cultured in fetal bovine serum. Cultures will be maintained for 21 days, and then cells will be stained with a live/dead viability kit. Stained samples will be imaged under a fluorescence microscope and the cell line proliferation or lack thereof will be determined to conclude cell line compatibility with the scaffold material.

Technology Validation

We plan to conduct technology validation in five phases outlined below:

Phase 1: Initial Research

- Chitin
    - Deacetylation
    - Functionalization
    - Characterization
- High Throughput Screening

    - Comonomer
    - Photoinitiator
- Resin Formulation
- Scaffold Printing
- Screening of Scaffolds for cell viability, adhesion, proliferation, and differentiation

Phase 2: Optimization of Resin Formulation

- Functionalization
- Scaffold Printing
- HTS of Scaffolds

Phase 3: Viability Analysis

- Texture analysis of scaffolds and scaffolds with cells against respective animal meat target
- Sensory analysis of scaffolds and scaffolds with cells against respective animal meat target

Phase 4: Partnerships

- Partnership with downstream processors/clients to collaboratively optimize the scaffolds and test against parameters mentioned in Phase 3 above
- The scale-up scaffold manufacturing process

Phase 5: Sustainable Chitin Sourcing

- Chitin reclamation via microbial fermentation using agro-waste as carbon source

It is important to note that activities in the phases are not necessarily chronological and may run simultaneously or interdependently based on needs. Overlap of phases is also to be expected depending on new hurdles discovered and progress made.

We have listed below the methods we will utilize for technology validation during research and product development:

- **Happy cells:** Total DNA quantitation, DAPI nuclei staining (to look at total cell number and even cell distribution, actin phalloidin cytoskeleton staining (cell attachment and spreading) (Ben-Arye et al., 2020).

- **Texture analysis:** Texture comparison will be conducted using classical tests used in the meat industry, such as Kramer Shear Cell and Warner Bratzler shear blades, to compare against animal targets (Freeman, n.d., Schreuders et al., 2021). In addition, tensile strength and TPA (Texture Profile Analysis or Double Compression Tests) will be used to support sensory analysis.
- **Imaging:** Microscopic characterization using SEM, AFM, and MRI to study scaffold structure and monitor cell growth and distribution
- **Degradation analysis:** Enzymatic degradation of printed hydrogel constructs characterized by mass change over time and analyzed by optical imaging
- **Sensory analysis:** Triangle taste test by semi-trained sensory panelists of cooked meat on 3D printed scaffolds against respective animal meat target

Cost Analysis

Overall, the cost of our solution is significantly less expensive (a few cents per kilogram) compared to other alternative meat and scaffold companies that currently exist. There is also the added benefit of operating in a circular bioeconomy: redirecting a waste product from landfills. The first step is to analyze the cost of chitin. As a waste product, chitin in its unrefined form can be purchased for $0.10 per kilogram (Hancocks, 2020). Industrially refined chitin derived from seafood waste is around $4 per kilogram, and its price can be decreased further in bulk purchases from the manufacturer (Blueweight Biotech LLC, n.d.). Domestically produced chitin can be processed from seafood waste using acid-base reactions for a cost of roughly $8 per kilogram, a relatively low-cost option (I.M.C.N. et al., 2018). Additionally, fungal chitin purchased from a licensed retailer is approximately $48 per kilogram. Given that chitin will be the primary backbone polymer of our hydrogel, we assume that chitin will be at concentrations of 1-5% at maximum swelling, which is standard for these types of hydrogels. Thus, these scaffolds' backbone raw material cost will only be a few cents per kilogram. We also plan to explore the chitin reclamation process using microbes, which may change our cost of chitin in the future. Based on current research estimates, we anticipate such costs to be roughly $8 per kilogram (I.M.C.N. et al., 2018), a relatively low-cost option.

For the printing aspect of our solution, there are two components to consider: the printer and the resins needed. According to Formlabs, the average professional SLA printer costs about $11,000, but enterprise/industrial printers can cost between $20,000 and $100,000 ("*How much does a 3D printer cost?*", n.d.). We anticipate the cost of food-safe SLA printers to be the same cost as conventional ones. However, the cost of printers is expected to decrease over time as consumer demand increases and technology evolves.

Additionally, the other primary cost to consider for the production process is sterilizing the manufacturing space and the meat. Conventional irradiation equipment can range anywhere from $150,000-$400,000 (Blair Polin, 2015), but cheaper options include using third-party E-beam or gamma radiation providers for sterilization. Irradiators in the chicken poultry industry around from 0.01-0.05 cents per pound, and costs decrease as the pounds of meat processed increases (Morrison, 1989). For pork, the cost would be closer to 7 cents per pound, decreasing as the amount of meat processed increases (Cardona et al., 2003). Such prices will be similar for the proposed solution.

Based on these anticipated costs, the lower end of the cost for research and production for our first year of research and production will most likely be close to USD 2 million ($1,923,001).

- Chitin Sourcing: $4 * 100,000 kg = $400,000
- Food-grade SLA printers: $11,000 * 3 printers: = $33,000
- Supplies to create Food-Grade Resins: $149 * 10,000 Liters = $1,490,000
- Irradiation equipment: $0.0001 * 10,000 lbs processed = $1

The higher end of costs for our first year of research and production will be closer to roughly USD 7 million ($7,100,005).

- Chitin Sourcing: $48* 100,000 kg = $4,800,000
- Food-grade SLA printers: $100,000 * 3 printers: = $300,000
- Supplies to create Food-Grade Resins: $200 * 10,000 Liters = $2,000,000
- Irradiation equipment: $0.0005 * 10,000 = $5

This preliminary cost analysis is limited to material cost and does not include any additional costs such as research space, employee's compensation or any unexpected expenses that may arise during the initial experimentation and research phase. Costs are also expected to change as we continue to research ways to reclaim chitin from seafood waste and other sources more efficiently.

Discussion of the Status Quo

Currently, "meatless" meats are a burgeoning industry, with companies such as Impossible Burger and Beyond Meats working on products based on plant proteins (Piper, 2019). However, one of the primary issues with this strategy is that such ingredients and techniques make it difficult for these companies to produce whole cuts of meat as opposed to ground patties. As a result, many companies, such as Redefine Meat and MeaTech 3D, have been making major strides in 3D printed meat. These companies employ extrusion-based printing techniques to produce various types of structurally complex meat, ranging from sausages to steaks. Because of the current, low-resolution extrusion-based printing technology, it is challenging to make the meat cuts appear realistic. This artificial look may be unpleasant to some consumers, impacting overall sales and reception of the product. However, our proposed SLA printing mechanisms utilize lasers, allowing for cleaner and more precise scaffold features for cells to be deposited onto, differentiate, and grow on. As a result, the final product will more closely resemble meat than current extrusion-based printing methods. The addition of animal cells that can differentiate in scaffolds also helps these whole cuts of meat to stand out as higher in nutritional value than plant-based counterparts.

Sustainability Analysis

Our proposed solution is more sustainable than both conventional meat processing methods and current 3D printed meat methods. Currently, agriculture and resources used for animal production consist of a substantial subset of greenhouse gas emissions, with estimates of nearly 17000 Tg CO_2 (Xu et al., 2021). Animal-based products play a looming and unchecked role in global climate change. The transition towards animal-less meat products has significant benefits on the environment as a result of using fewer resources

and water than conventional meat production. For example, Impossible Burger notes that its soy patties use about “87 percent less water, takes 96 percent less land, and has 89 percent lower greenhouse gas emissions than a beef burger” with Beyond Meat also making similar claims (Hayek and Dutkiewicz, 2021). Our solution would have similar environmental impacts as Impossible Burger and Beyond Meat and reduce waste in the food production industry.

Figure 11. Shrimp shell waste stocked in Odisha, India *(The New Indian Express)*

Companies innovating in the 3D printed meat industry have been working primarily with plant proteins or stem cells/live cultures. For example, Redefine Meat primarily uses plant-based proteins from grains, legumes, and soy as well as some fats from plants (Ben-David, 2021). Additionally, MeaTech 3D relies on bovine stem cells to create their bio-ink (Carrington, 2021), while Aleph Farms usually uses live cell cultures to grow their steaks using cultivators to transform these cell cultures into muscle cells and create the final meat product (Aleph Farms, n.d.). As a viable alternative, we propose using chitin in seafood waste as a potential source of ink used in the printing process. Seafood waste is a heavily underutilized resource of chitin and chitosan, the primary material used in the chitin-based hydrogel cell scaffolds. Currently, nearly six to eight million tons of seafood

waste is discarded annually (ScienceDaily, 2020). However, a large amount of such seafood waste that enters the environment is unprocessed and untreated, which has the potential to cause environmental damage (Santos et al., 2020). By utilizing seafood waste in the printing process, we can dramatically save costs and reduce the amount of pollution and waste entering the environment. Additionally, chitin is an abundant component in the cell walls and mycelium of fungi, thus making fungi another viable source of chitin (Lenardon et al., 2010). As a result, we do not have to rely solely on the seafood industry's waste for chitin during the manufacturing process. From a sustainability perspective, our solution of using chitin-based hydrogel scaffolds has a more positive impact on the environment than current methods that rely on plant proteins or animal cell cultures for "bio-inks."

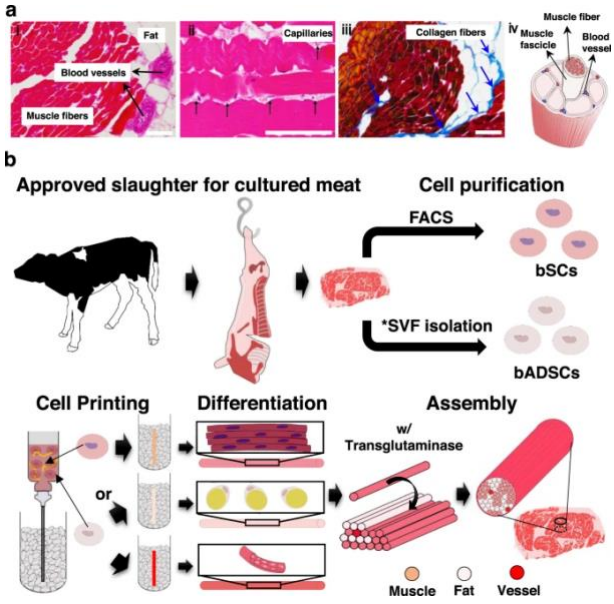


Figure 12: Current production methods of 3D printed meat using the extrusion method (*Kang et al., 2021*)

Feasibility and Applicability

Our solution is something that can easily be implemented into the current industry. Chitin is already viewed as a viable biomaterial by some, as a handful of companies are currently exploring its ability to function as a scaffolding substrate. For example, Excell is

currently experimenting with using fungal mycelium, which contains chitin, as a possible scaffolding material in cell-cultured meat.

Other than Excell, several companies have also worked on extracting chitin from seafood waste for different uses. For example, CuanTec and CruzFoam recycle chitin from seafood waste to produce eco-friendly packaging. This process of cleaning and recycling chitin from seafood waste can easily apply to other products, especially to meat substitutes. However, none of these companies have explored the use of chitin as a base for the edible photopolymerizable scaffold.

SLA printing is a recent innovation with tremendous potential to create realistic meat without the same resources as conventional production methods. Our proposed SLA resin would be a viable method to explore 3D printed meat scaffolds, especially since 3D printing costs have reduced significantly over the years. In the late 1980s, the average SLA printer cost around $300,000 (Miller, 2016), while now, the average cost of a professional printer is around $11,000 (Formlabs, n.d.). Overall printing costs have decreased significantly and are projected to decrease more with time due to ongoing innovations in printing technology and increased demand for items produced by printers. The food industry will certainly benefit from this overall decreasing trend, making 3D printed scaffolds much cheaper than conventional counterparts.

Potential Risks and Challenges

Although there are many benefits to implementing 3D printed meat scaffolds, there are several risks and challenges associated with this proposal. The primary challenge is seeking regulatory approval from the U.S. Food and Drug Administration (FDA), the U.S. Department of Agriculture (USDA), and other international safety organizations to get this product to the market. For example, in 2019, FDA approval for Impossible Burger took nearly half a decade to ensure that all aspects of the production process were fully compliant with FDA regulations (Lucas, 2019). As a result of this approval, pathways have been set so that future companies innovating in the alternative meat space can easily gain approval within a few years. Additionally, more countries are slowly approving “meatless” meats, with Singapore being the first country to approve cultivated meat as of

December 2020 (Vance, 2022). As a result of increasing consumer demands and meat substitutes, FDA approval for such products will likely be a more streamlined process.

The sterilization of cultured meat is also an important factor to consider. Culturing cells always poses a risk of contamination; thus, FDA regulations are required to ensure meat safety and quality. Sterilization is of utmost importance when developing scaffolds for cultivated meat to ensure that the final product is as germ-free as possible. There are several viable options to ensure a sterile scaffold and production environment, including utilizing gamma and E-beam irradiation, heat for ultra-sterilization, and chemical compounds such as ethylene oxide, ethanol, hydrogen peroxide, and peracetic acid. Irradiation techniques using gamma rays are currently FDA approved and are used in various foods, including beef, pork, fruits, crustaceans, and other poultry products (Center for Food Safety and Nutrition, 2020). Heat sterilization is a common technique to preserve meat and ensure its safety since it destroys any microbial cells, spores, and enzymes that may be present on the meat (Li et al., 2020). Using various cleaning compounds, such as hydrogen peroxide and acetic acid, on surfaces in the processing facility significantly reduces E. coli cultures on meat (Bell et al., 1997).

Another risk to be aware of is the food safety of our SLA resin. SLA printing relies on resins to cross-link during the photopolymerization process, yet many monomers that have the capabilities to cross-link currently used in printing are not food grade or food safe (Formlabs, n.d.). However, there are plenty of known food-grade monomers and resultant polymers that are used in food applications as additives and coatings, that will be explored and utilized in our proposed solution. The use of such polymerizable food-safe monomers in resins is critical in ensuring that the end product is not only safe but also looks presentable when creating the final meat product. In addition to food-safe resins, some people experience sensitivities to common antigens present in chitin extracts, which may cause serious allergic reactions in some (Burton and Zaccone, 2008). Thus, it is essential to consider such risks when performing high throughput screening and during the testing stage.

Overall, our proposed solution is feasible and applicable to the current industry and a very sustainable and eco-friendly alternative to conventional meat products available in the status quo. All the possible risks and challenges associated with chitin-

based scaffolding are things that can be addressed and overcome in the long term, thus making it a very viable product in the cellular agriculture market.

Time Scale

In 2020, roughly 50-70 young and old companies were operating in the cellular agriculture industry according to the 2020 State of The Industry report from GFI (Byrne and Murray, 2020). Most of these companies focus on full-stack development, while only three companies are actively pursuing scaffold development. We strongly believe that the full-stack model will not be viable for commercial penetration of cultivated meat and sooner or later, many of these companies will outsource part of their focus areas, especially scaffolding, for time and cost efficiency reasons. This is the market we will be capturing.

To achieve a 10% market share of scaffolds used in cellular agriculture production (roughly translating to 5 serviceable obtainable clients) within the next 3 - 5 years, our timeline estimates are listed below:

Phases	Description	Timeline
1	Initial Research	Year 1 & 2
2	Optimization of Resin Formulation	Year 1 & 2
3	Viability Analysis	Year 2 & 3
4	Partnerships	Year 3 & 4
5	Sustainable Chitin Sourcing	Year 5

Scale-Up Challenges:

We foresee the following as some of the major challenges to a scaleup:

- Efficient high throughput screening of functionalized chitin, comonomer, photoinitiators
- Commercial availability of identified comonomer and photoinitiator
- Speed of photopolymerization
- Yield and speed of commercial SLA printers

Conclusion

Cellular agriculture is an industry that can revolutionize not only the food industry but the world. Meatless meats have numerous benefits, ranging from environmental benefits to humanitarian ones. Our transition towards more animal-free food alternatives can significantly benefit our environment by reducing greenhouse gas emissions and redirecting valuable resources used to raise livestock towards ecological preservation. Cultured meats are viable alternatives to feed our global population, as it contains the same nutritional profile as conventional meat, without the same ethical and environmental issues of the seafood, poultry, pork, and beef industry.

The additive manufacturing industry has been the backbone of many life-changing projects that also have the power to revolutionize our world for the better. From innovations in printing organs from tissue cells (Paul et al., 2018) to entire houses (Bellamy, 2022), this technology can help mitigate and solve urgent crises that society faces. The 3D printing industry is making far-reaching breakthroughs, and within the next few years, it can help make the world a better place through its continued application in cellular agriculture.

We combined the best of 3D printing technology with the potential of a burgeoning industry by creating a one-of-a-kind product. Our low-cost, sustainable chitin-based scaffold essentially turns a waste product into a resource that can help mitigate the many dire crises our world faces, from greenhouse gas emissions to world hunger. Our solution also has the capabilities to evolve as needs change and as the available resources change over time. For example, we can source chitin and chitosan from various abundant and low-cost sources, and we can easily tailor our resin formulation to work with alternative polysaccharides with the ability to create hydrogels. Additionally, our 3D printed scaffolding mechanism can also continually improve as SLA printing continues to improve along with the development of newer food-grade monomers and photoinitiators.

Large problems require bold solutions, which is precisely what our proposal is. We believe in utilizing the Earth's abundant natural resources at the interface with cutting-edge material manufacturing technologies to solve the world's most pressing challenges. 3D printed scaffolds for cellular agriculture will create an ecologically sustainable and healthy planet for generations to come.

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Photos

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