The perception of in-vitro meat (IVM) by New Zealand consumers

By

Maya Murthy Malavalli

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Primary Supervisor: Professor Nazimah Hamid

Secondary Supervisor: Dr Ali Seyfoddin

ABSTRACT

Purpose- The purpose of the study was to investigate the perception of in-vitro meat (IVM) among New Zealand consumers and to understand their purchase and consumption behaviour using a customized conceptual framework developed in this study. In addition, preliminary trials for production of IVM using stem cells, biopolymers, edible 3D scaffolds and bioprinting technology were carried out as an alternative to traditional 2D cell culturing technique.

Methodology – An online survey questionnaire was created using Qualtrics software to understand the perception of IVM, based on the conceptual framework. Participants (n=206) were recruited personally to take part in the survey. The data collected in the survey were subjected to PLS-PM analysis. The conceptual framework was tested for validity, Goodness of fit (GoF), Cronbach's alpha. Whereas, the internal validity was assessed using Cronbach's alpha, KMO value, inter-item correlation values (β-coefficients) and p-values. All the analyses were carried out using R Studio 1.1.463 with R version 3.5.1 PLSPM package.

Findings- The findings suggest that variables such as environment and sustainability, health and safety, current purchase and consumption behaviour have a strong relationship and a robust effect on IVM purchase and consumption behaviour. Consumers' cultural beliefs had minimal influence on IVM purchase likelihood. Results in this study also indicated that most New Zealand consumers had neutral opinions in terms of engaging with IVM. Hence, if NZ consumers are educated about IVM and its potential benefits, then IVM will be more acceptable. Whereas, preliminary trials to produce 3D IVM using stem cells were unsuccessful due to damage and fungal contamination of the cell-laden scaffolds.

Research limitations – The online survey in this study was open to all consumers in New Zealand (NZ), however, the majority of the participants were young and educated consumers. Thus, the sample population may not be a representative of the general population. Furthermore, this study was focussed on New Zealander participants. It would be beneficial to have comparisons across participants from different countries.

Practical implications – This study fills the research gap by providing insights into the opinions of New Zealanders on IVM. The findings from our study will be beneficial for future IVM/ sustainable meat alternatives industry, as it provides information on in-vitro meat purchase and consumption behaviour based on New Zealand consumers opinion.

Originality/ value – This is the first study on New Zealand consumers that addresses the consumer's purchase and consumption behaviour towards IVM.

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ABBREVIATIONS

IVM = In-vitro meat

FBS = Foetal Bovine Serum

WTT = Willing to try

WTP = Willingness to purchase

PEG = polyethylene glycol

PLLA = poly- (L-lactic acid)

PLGA = polylactic-glycolic acid

PDMS = polydimethylsiloxane

PVA = poly- (vinyl alcohol)

GelMA = gelatin methacrylate / Gelatin Methacryloyl

HA = Hyaluronic acid

GLOSSARY

- Adipocytes - Adipocytes or fat cells are derived from mesenchymal cells which primarily form adipose tissue that stores energy as fat (Source: Wikipedia).
- Adult stem cells - Adult stem cells are undifferentiated cells found throughout the body that divide to replenish dying cells and regenerate damaged tissues.
- Bovine – a biological term for cow as it belongs to Bovidae family (Source: Wikipedia).
- Bioreactor – bioreactors are manufacturing units which provides a biologically favourable environment and supports the growth of organisms (Source: Wikipedia).
- Cell death – is the event of biological death of cells due to the natural process of the cell cycle (Source: Wikipedia).
- Covalent bond – are the chemical bond formed by the sharing of electrons between two atoms (Source: Wikipedia).
- Dedifferentiation – the process by which specialized cells regress to simpler stem cells (Source: Wikipedia).
- Differentiation - the process by which cells, tissues and organs acquire specialized features during embryonic development (Source: Wikipedia).
- Dedifferentiated cells – cells which have undergone dedifferentiation.
- Differentiated cells – cells which have been differentiated to perform specialized functions.
- Embryonic stem cells - stem cells derived from the undifferentiated inner mass cells of a human embryo.
- European consumers – Consumers in the European countries or European Union.
- Fibroblast – are the cells which form extracellular matrix, collagen and connective tissues. These cells are also responsible for wound healing (Source: Wikipedia).
- Induced pluripotent stem cells (iPSC) - are a type of pluripotent stem cell that can be generated directly from adult cells (Source – Wikipedia).

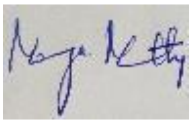
- Ionic bond – bonds formed between a positively charged ion and a negatively charged ion and brings about a transfer of ions among atoms (Source: Wikipedia).
- Mechanotransduction - a process through which cells sense and respond to convert mechanical stimuli into electrochemical activity (Source: Wikipedia).
- Multipotent - are cells that have the capacity to self-renew by dividing and to develop into multiple specialised cell types present in a specific tissue or organ (Source: Wikipedia).
- Myoblast – is a cell that is responsible to form muscle cells (Source: Wikipedia).
- Myotube – these are formed by the fusion of myoblasts into multinucleated myofibrils.
- Myofiber – these are units of muscle fibres, which are formed by the fusion of myofibrils (Source: Wikipedia).
- Myogenesis – It is the formation of muscle tissue during embryonic development (Source: Wikipedia).
- Myocyte – are long tubular cells formed by myoblasts which help in the formation of muscles (Source: Wikipedia).
- Ovine – pertaining to sheep (Source: Wikipedia).
- Pluripotency – the ability of stem cells to develop into all three primary germ layers during embryonic development (Source: Wikipedia).
- Pluripotent stem cells - cells that have the capacity to self-renew by dividing and to develop into the three primary germ cell layers of the early embryo (Source: Wikipedia)
- Porcine – pertaining to pigs (Source: Wikipedia).
- Proliferation – is the process of increase in the number of cells by cell division (Source: Wikipedia).
- Shear stress - Shear stress is defined as a force per unit area, acting parallel to an infinitesimal surface element (Source: Wikipedia).
- Stem cells - an undifferentiated cell of a multicellular organism which is capable of giving rise to indefinitely more cells of the same type (Source: Wikipedia).
- Transfection – it is the process of deliberately introducing naked or purified nucleic acids into eukaryotic cells. (Source: Wikipedia).

- Transdifferentiation - is the conversion of a cell type present in one tissue or organ into a cell type from another tissue or organ without going through a pluripotent cell state.
- Western Countries – Countries in the west, such as the United States of America (USA) and Canada.
- Western Diet – Diets which have high intakes of red meat, sugar, fat, salt, and refined grains.

Attestation of Authorship

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, 'The perception of in-vitro meat (IVM) by New Zealand consumers' contains no material previously published or written by another person (except where explicitly defined in the acknowledgements), nor material which to a substantial extent has been submitted for the award of another degree or diploma of a university or other institution of higher learning.

Name: Maya Murthy Malavalli

Sign: 

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1. Chapter 1. INTRODUCTION

In-vitro meat is a type of meat that is produced using animal cells, under laboratory conditions (Post, 2012). It is a recent development in the sustainable food industry, which is still in infancy stages. In-vitro meat (IVM) works on the principle of cellular agriculture[1], where stem cells are extracted from donor animals through biopsy. The extracted cells usually belong to embryonic stem cells (ESC) (Catts and Zurr et al., 2002a), adult stem cells (ASC) (Benjaminson et al., 2002; Williams, 2012), mesenchymal stem cells (MSC) (Jossen et al., 2014; Verbruggen et al., 2018) or induced pluripotent stem cells (iPSC's) (Benjaminson et al., 2002). These cells are cultured either by scaffolds or self-organising techniques (Benjaminson et al., 2002) or are cultured in a sterile bioreactor. The extracted cells, under favourable conditions, undergo cell proliferation and differentiation to form myofibers, which eventually form muscle tissues. These muscle tissues combine to form skeletal muscles, which can be harvested as edible meat.

The commercial success of IVM strongly depends on consumers' perceptions on IVM. In fact, perceptions determine whether IVM will be acceptable or rejected. Therefore, it is essential to understand consumer perception. Moreover, the information on consumer perception will be beneficial for future IVM companies to generate effective marketing strategies and newer product based on public perception. Due to these reasons, there has been extensive research on consumer perception of IVM in the past few years.

Most of the studies on IVM perception have focused on the American consumers (Bryant and Dillard, 2019d; Bryant et al., 2019c; Wilks and Phillips., 2017; Wilks et al., 2019) and European countries (Hocquette et al., 2015; Verbeke et al., 2015). There has however been few studies with Chinese and Indian (Bryant et al., 2019c), New Zealand (Tucker, 2014), Australia (Lupton and Turner, 2018), and Brazil and Dominican Republic (Gómez-Luciano et al., 2019) consumers.

Consumer perception on IVM was generally found to be more positive among European consumers compared to US consumers. European consumers were more interested in engaging with IVM, with 52% of the Netherlands consumers definitely

[1] Cellular agriculture refers to the production of animal derived products through cell culture, with combination of tissue engineering, biomedical sciences and biotechnology (Source: Wikipedia).

willing to try IVM (Flycatcher, 2013), 68% of the United Kingdom consumers willing to eat IVM (Guardian, 2012) and 44% of Italian consumers willing to buy IVM (Mancini and Antonioli, 2019). In comparison, only 31.3% of US consumers were willing to try IVM (Wilks and Phillips, 2017), 31.3% of consumers were willing to eat IVM as a replacement to traditional meat (Wilks and Phillips, 2017), and only 19.8% of the US consumers were willing to buy IVM (Bryant et al., 2019c). These findings showed that Europeans perceived IVM as being more acceptable. Consumers in the USA were more sceptical about IVM and perceived it as being less acceptable. Lower meat attachment and higher food neophobia among US consumers leads to decreased IVM acceptance (Bryant et al., 2019c). Acceptance of IVM by Europeans consumers (Flycatcher, 2013; Hocquette et al., 2015; Mancini and Antonioli, 2019; O'Keefe et al., 2016; Shaw and Mac Con Iomaire, 2019; Verbeke, Sans and Van Loo, 2015b) was due to increased IVM familiarity brought about by the unveiling of IVM burger in London, 2013.

There are more concerns about IVM in terms of taste (Wilks and Phillips, 2017), unnaturalness (Laestadius and Caldwell, 2015; Marcu et al., 2015; Verbeke et al., 2015; Welin, 2013; Wilks and Phillips, 2017), disgust (Pluhar, 2010; Siegrist et al., 2018; Van der Weele and Driessen, 2013; Verbeke et al., 2015), price (Mancini and Antonioli, 2019), perceived health and safety risk, and technology involved in IVM production (Verbeke et al., 2015). IVM acceptance on the other hand is influenced by environmental (Gómez-Luciano et al., 2019) and animal welfare issues (Hocquette et al., 2015), ethical reasons (Wilks and Phillips, 2017), and perceived health benefits (Verbeke et al., 2015).

New Zealand consumers are considered as one of the highest meat consumers in the world. In addition, NZ is economically invested in the traditional meat industry. This study seeks to understand the perception of IVM by New Zealand consumers, consumers' willingness to try/buy IVM and their reasons for acceptance/rejection towards IVM.

1.1 Purpose and objective

This study seeks to understand the perception of IVM by New Zealanders as these findings will add valuable insights for future IVM markets. Furthermore, the information gained through this study will also help in creating better marketing strategies for IVM companies in the future.

Main research questions in this study are:

What do New Zealand consumers think of IVM? Will they accept or reject IVM? What are the reasons for their rejection and acceptance? Would they be willing to buy/try it? Are their perceptions influencing their purchase and consumption behaviour?

1.2 Hypothesis

Literature has shown that consumers' perceptions on IVM have a strong effect on IVM purchase and consumption behaviour. In fact, positive perceptions on IVM has been linked to higher purchase likelihood. For example, consumers who believe that IVM is beneficial for the environment and animal welfare, are more likely to buy IVM (Gómez-Luciano et al., 2019; Shaw and Mac Con Iomaire, 2019; Wilks and Phillips, 2017) compared to those who think IVM is a waste of resources (Laestadius, 2015a).

On the other hand, research shows that IVM purchase intentions are influenced by many factors such as, consumers' environmental and welfare awareness (Wilks and Phillips, 2017), their openness towards sustainable alternatives (Bryant et al., 2019c), their awareness of IVM and its sustainability (Mancini and Antonioli, 2019), IVM sensory qualities (Hocquette et al., 2015; Mancini and Antonioli, 2019), and IVM health and safety (Hocquette et al., 2015; Mancini and Antonioli, 2019).

Consumers with higher environmental and welfare awareness tend to have higher IVM purchase likelihood, due to IVMs environmentally and animal-friendly nature. Consequently, these consumers have higher purchase intentions compared to others (Bryant et al., 2019c). Hence consumers with higher environmental and welfare awareness definitely have higher openness towards sustainable alternatives (plant-based alternatives or animal-based). Subsequently, such consumers, environmentalists and welfare activists have higher IVM purchase likelihood (Bryant et al., 2019c).

Consumers with higher awareness on IVM and its potential benefits find IVM more acceptable and tend to have a higher purchase intention towards IVM. This was confirmed by many researchers (Bryant and Dillard, 2019d; Flycatcher, 2013; Verbeke et al., 2015b), indicating that purchase intentions are linked to many factors. Consumers who have positive perceptions on sustainable alternatives and agree that IVM is a sustainable alternative tend to have higher purchase intentions (Bryant et al., 2019c; Mancini and Antonioli, 2019), as opposed to the pro-traditional meat individuals (Hocquette et al., 2015; Laestadius, 2015a). Consumers with strong sensory preferences and health and safety concerns tend to have reduced purchase intentions towards IVM (Laestadius, 2015a; Shaw and Mac Con Iomaire, 2019) since IVM still requires advancement in taste and safety aspects. Consumers with higher meat consumption tended to have lower inclinations to IVM (Tucker, 2014).

Based on the findings described, the hypotheses of this research are as follows:

H1: Consumers' views on environment & sustainability has a significant effect on IVM purchase and consumption behaviour.

H2: Consumers' views on health and safety has a significant effect on IVM purchase and consumption behaviour.

H3: Consumers' cultural beliefs has a significant effect on IVM purchase and consumption behaviour.

H4: Consumers' current purchase and consumption behaviour has a significant effect on IVM purchase and consumption behaviour.

Null hypothesis / H_0: Consumer's views on environment and sustainability, health and safety, and cultural beliefs. as well as their current purchase and consumption behaviour do not influence IVM purchase and consumption behaviour.

This study will test and confirm if the above hypotheses (H1/ H_0) hold true for New Zealand consumers. The hypothesis is based on the conceptual framework used in this study, as seen in Figure 3-1.

2. Chapter 2. LITERATURE REVIEW

2.1 NEW ZEALAND MEAT INDUSTRY

The New Zealand meat industry plays a critical role in the country's economy, as it is one of the strongest markets in the export industry (Beef and Lamb NZ, 2017). The meat industry generates 27% of export revenue and fetches an income of over $8.5 billion (New Zealand Dollars) as of 2018 (Meat Industry Association, 2019b). In addition, reports show that the global export value growth rate of NZ meat industry is about 6% CAGR (compound annual growth rate) (Ministry of Business, 2017), as seen in Figure 2-1. These statistics indicate the importance of the meat industry to the New Zealand economy.

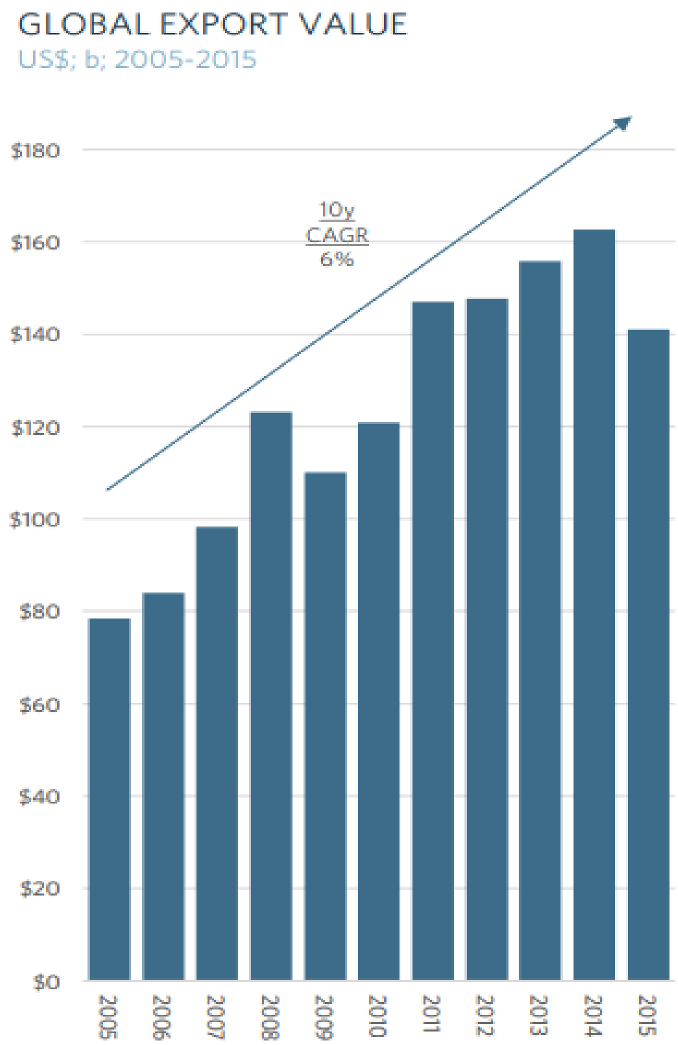

Figure.2-1 New Zealand meat industry's annual export trade growth rate for red meat (Ministry of Business, 2017)

The New Zealand meat industry produces almost half a percent (322m tonnes) of the total world meat supply (Ministry of Business, 2017). 85% of NZ meat is exported to 120 overseas countries (BusinessNZ, 2019). Most venison meat (95%) is exported to other countries (Meat Industry Association, 2017), and generates a revenue of over $150 million NZD every year (Deer Industry, 2018). The majority of lamb meat (92%) exported to Gulf states, USA, UK, Germany (Meat Industry Association, 2017), and China (Ministry of Business, 2017). Whilst, New Zealand produced sheep meat is mainly exported (92%) (Beef and Lamb NZ, 2017) to China (39%) (Fortune, 2019), United Kingdom (22%) (Meat Industry Association, 2017) and the European Union (EU) (30%). However, exports to the European market has significantly declined (Meat and Livestock Australia, 2018) due to unknown reasons. The NZ based beef industry, although a minor global producer (1%) (Ministry of Business, 2017), generates $2.8 billion NZD per year, makes up 7 % of the global trade. 82% of beef to countries like United States of America (USA), Japan and Korea (Meat Industry Association, 2017). These statistics confirm that NZ has high economic investments in the meat industry.

New Zealand is one of the countries with the highest number of meat consumers in the world (Pereltsvaig, 2013; The Economist, 2012). The most commonly consumed meat is poultry (39.4 kilograms/capita) followed by beef and veal (11.9 kilograms/capita), pig meat (18.9 kilograms/capita), sheep meat (4.7 kilograms/capita) (Beef and Lamb NZ, 2013). The above statistics indicate that New Zealanders have a higher dependency and attachment on meat and its products.

2.1.1 Meat alternatives

As meat and dairy production are reaching their limits in New Zealand with respect to sustainability, more consumers are looking for meat alternatives. A few NZ owned businesses such as Fonterra and Air New Zealand have exhibited their strong support towards IVM and plant-based meat alternatives in the past few years (Communications, 2018; Hutching, 2019). There are also increasing NZ owned meat alternative companies such as Sunfed, Craft mince, Vegie Delights, Bean Supreme, Amino Mantra, Alt Meat Co, Tonzu and Soy Works, which offer plant-based meat alternatives (Vegan society NZ 2019). Most of the plant-based meat alternatives also suffer from concerns such as disgust, poor acceptance and palatability problems. However, despite the concerns, there still exists a place in the market for meat alternatives. This could mean that IVM could have a niche market in the industry.

Research has shown that New Zealand consumers are concerned about sensory attributes, price, convenience and health impacts of food products (Prescott et al., 2002). These concerns may influence their perceptions of IVM, which being a novel product has drawbacks such as unnaturalness, taste and health risks that require improvements, especially on sensory attributes.

2.2 CONSUMER PERCEPTION OF IN-VITRO MEAT (IVM)

Consumer perception of novel food products depends on factors such as effects on health, nutrition, safety, affordability, food preference, personal beliefs, cultural identities, religious views, sensory quality and nature of the product. Based on these factors, consumers either accept or reject the food product, as is the case for IVM.

Although a considerable amount of literature has been published on the consumer perception of IVM, most of these studies are empirical work, rather than quantitative research.

Most studies have reported consumer concerns on IVM rather than acceptance. Furthermore, perceptions are varied and ambiguous in some studies. For example, some researchers have stated that IVM will remain a niche market (Stephens, 2010), while other researchers think that IVM is predisposed to consumer reluctance before it can be accepted. These contradictory findings are likely due to three major concerns – consumer concern, personal concern and ethical concern. Firstly, consumer concerns such as attitudinal differences, meat attachment, food neophobia affect the perception of IVM. Secondly, personal concerns such as unnaturalness, disgust, palatability problems, price concerns, perceived health and safety risks, sociocultural beliefs and lack of familiarity influence the perception of IVM. Lastly, ethical concerns of IVM on animal welfare and environment, ethical concerns on the technology itself and its fair trade, ethical concerns on the unnaturalness of IVM and its possibility of animal extinction and food inequality. The concerns mentioned above will be discussed in detail in the section 2.2.1

2.2.1 Consumer Concerns

2.2.1.1 Attitudes

Consumer attitudes towards food technologies are critical as it determines acceptance or rejection. Attitudes such as willingness to try (WTT), willingness to buy

(WTB) and willingness to eat (WTE) are most the researched aspects of IVM to understand its potential market.

Inarguably, the commercial success of a food product lies in the consumer perceptions and the number of consumers willing to try (WTT) and willing to purchase/ buy the product (WTP / WTB respectively), but mere consumers' willingness to try the product does not imply likelihood to purchase. Several studies have been carried out to understand consumers' willingness to try IVM, both in the US and other European countries (Bryant et al., 2019c; Slade, 2018; Wilks and Phillips, 2017). However, the findings vary due to the nature of the study, research design, methodologies, data collection and differences in the sample population.

2.2.1.1.1 Willingness to try (WTT)

United States of America market (US)

Wilks et al. (2017) reported that only 31.1% (n=673) of US consumers were definitely willing to try (WTT) IVM, and 34.2% were probably willing to try IVM. This is likely due to a higher percentage of meat-eating consumers (88%) with higher education (44.6%). Furthermore, Bryant et al. (2019a) suggested that the perception and WTT IVM depended on US consumer's meat attachment and food neophobia levels. Food neophobia and meat attachment consumer concerns are described in detail in section 2.2.1.2. On the contrary, about 64.6% (n=480) of US consumers were probably or definitely willing to try IVM, and 18.4% were probably or definitely unwilling to try IVM (Bryant and Dillard, 2019d). These differences in WTT results may partly be influenced by the positively framed pre-test information on IVM provided to consumers.

European markets

The willingness to try (WTT) IVM among Europeans consumers also varied, with about 52% (n=1296) of Dutch consumers were willing to try IVM. The higher WTT percentages was a positive effect brought about by the provision of information on IVM (Flycatcher, 2013). Similarly, 67% (n=180) Belgian consumers and 54% (n=525) of Italian consumers (Mancini and Antonioli, 2019) were willing to try IVM. In addition, 43% of Belgian (Flanders) consumers were surely willing to try IVM and 51% were willing to try it (Verbeke et al., 2015b) when consumers were informed of IVM's environmental benefits. The higher WTT is possibly due to the higher number (71%) of consumers under the age of 25.

In general, a unique pattern was observed in the WTT IVM, in the USA and European markets. European consumers have a higher WTT IVM compared to the USA (Flycatcher, 2013; Mancini and Antonioli, 2019; Verbeke et al., 2015b; Wilks and Phillips, 2017). Such results are likely due to higher levels of meat dependency and meat attachment among European consumers, which leads to the acceptance of alternative meat such as IVM. This was brought about by the unveiling of an IVM burger in London, 2013. Lower WTT IVM among US consumers are linked to higher levels of food neophobia (Bryant et al, 2019d).

2.2.1.1.2 Willingness to buy (WTB)

United States of America market (US)

Many studies have explored consumer willingness to buy IVM (WTB) as it is essential for the establishment of potential markets. On this note, a study on US consumers (Bryant and Dillard, 2019d) showed that 49.1% (n=480) of consumers were probably or definitely willing to buy IVM regularly. These findings are in line with another online survey with US consumers that reported about 40% (n=1000) of consumers were willing to buy (WTB) IVM (Surveygoo, 2018). Similarly, Bryant et al. (2019), found that only 29.8% (n=987) of US consumers were willing to buy IVM. Whereas, Wilks et al. (2017) showed that 79% of the US consumers were not interested in buying IVM because of its taste or appeal. On the other hand, Slade (2018) from Canada, pointed out that the willingness to buy IVM burgers among Canadian consumers were found to be only 13% (n=533), despite informing consumers that IVM was analogous to traditional meat alternatives in terms of taste, nutritional and health safety aspects.

From the results, it is evident that some studies have higher WTB than others, it is likely due to the sample population characteristics, as some studies have a higher number of female consumers' (51.4%) (Bryant et al., 2019c), and older consumers' (>90%) (Slade, 2018). Besides, other reasons for reduced WTB are techno-scepticism (Dilworth and McGregor, 2015) and concerns on the unnaturalness of the product (Verbeke et al., 2015).

European markets

Many authors have investigated European consumers' willingness to buy (WTB) IVM (Hocquette et al., 2015; Mancini and Antonioli, 2019). Hocquette et al.

(2015) reported that only 9.2% (n=865) of French consumers were willing to buy IVM, with 26.2% (n=865) undecided, and the majority of consumers (64.5%) were not willing to buy IVM. In contrast, about 44% (n=525) of Italian consumers were willing to buy IVM (Mancini and Antonioli, 2019). Such differences in WTB can be linked to the sample population, as some studies recruited highly educated consumers and meat industry professionals (Hocquette et al., 2015) who are pro red meat. Besides that, reduced WTB can also be linked to dietary preferences and common repugnance towards food technology (Mancini and Antonioli, 2019). Moreover, reduced WTB can also be because of meat attachment and food neophobia, this will be further discussed in section 2.2.1.2.

Emerging eastern markets

Much importance has been given to perception of IVM among US consumers but have been less explored amongst Asian consumers. The willingness to buy (WTB) IVM among Indian and Chinese consumers are about 48.7% (n=1024) and 59.3% (n=1019) respectively (Bryant et al., 2019c). However, the high WTB rates in China and India could be attributed to the skewed sample population, which consisted of well-educated, urban consumers with high-income. In addition, differences in WTB may also be due to higher meat attachment seen in Asian countries compared to other countries (Bryant et al., 2019c). Essentially, higher meat attachment would result in higher WTB, but this may not be true for IVM purchase and acceptance.

Consumers from other parts of the world have also shown lower WTB IVM. Only 19.2% (n=817) International consumers (consumers from North America, Africa, China and other Asian countries) were willing to buy IVM (Hocquette et al., 2015).

2.2.1.1.3 Willingness to eat (WTE)

United States of America market (US)

Consumers' willingness to eat (WTE) IVM has been studied by many researchers, as it is vital to understand IVM acceptance. The WTE IVM among consumers have been ambiguous. In general, there was minimal interest but more hesitance when eating IVM.

According to Bryant et al. (2019c), 25% of the US (n=987) consumers showed reluctance towards WTE IVM, and this aversion may arise from meat attachment and dietary preference. A recent study showed that 80% (n=1001) of US consumers were hesitant about eating IVM (Griggs, 2014), and this was attributed to a higher number of elderly consumers recruited in the study who were not open to novel technologies. On the other hand, some researchers explored WTE IVM as a replacement to traditional meat. Results showed that 31% (n=673) of the US consumers were willing to eat IVM as a replacement, while the remaining 30% of the consumers were unwilling (Wilks and Phillips, 2017). There are many reasons for such varied results. Firstly, there are differences in the study design and methodologies used. Secondly, the nature of consumer sample population may have influenced the results. Thirdly, it could be due to differences in the way the IVM concept was explained and the way the questions were framed, which might have altered the results. Lastly, the usage of different measurement scales and analysis may have also led to the differences. Although these results may not be accurate, they give beneficial information for future studies,

European markets

A web-based survey showed that only 18% (n=1000) of consumers had WTE IVM (Surveygoo, 2018). However, the nature of the sample population is unknown as it is an online survey with not much information. Whereas, Hocquette et al. (2015) showed that only 5-8% (n=865) and 7-11% (n=817) of French and International consumers respectively, were willing to eat IVM (Hocquette et al., 2015). However, these findings could be biased as the study involved meat industry professionals, who may have minimal interest in IVM. Besides, the survey was translated into French. Hence, the credibility of the survey results may have been lost in the process of translation, so it is advisable to consider these results with caution.

In contrast to the above findings, an internet poll on UK consumers shows that 68% of the consumers were interested in eating IVM (Guardian, 2012). However, not much information is available on this poll, but such results may have been influenced by the familiarity created by media before IVM burger unveiling event.

Emergent markets

Interestingly, most Asian countries have slightly lower willingness to eat (WTE) IVM as reported by an online survey, such as 34% Thai (n=1007), 26% Chinese (n=1007) and 25% Malaysian consumers (n=1007) are willing to eat IVM (YouGov, 2018). In contrast to the above statistics on WTE, a majority of 52% Vietnamese (n=1007) were interested in eating IVM but there was not much information available on Malaysian, Vietnamese, Chinese or Thai consumers perception towards IVM (YouGov, 2018).

In contrast, another study showed that Chinese consumers were most inclined towards IVM compared to other consumer groups (Bekker et al., 2017). However, the statement above should be considered with care since it is not a representative of the general population as it only involved 30 university students. Unfortunately, there is no detailed literature on the perception of IVM by other consumer groups, such as the African continent consumers to give more accurate insights.

Despite the interests in trying/buying and/or eating, there are also reports of consumer rejection. For example, about 9% (n=180) of consumers from Belgium (Flanders), completely rejected the idea of IVM (Verbeke et al., 2015b), the actual reasons for rejections are unknown, but this might be due to disgust and concerns on unnaturalness, as previously observed by Verbeke et al (2015). Similarly, 55% of New Zealand consumers (n=69) completely opposed the idea of IVM becoming a part of the diet, but these results could be affected by sample population, mainly consisting of women and elderly consumer groups (Tucker, 2014). Furthermore, rejections may have originated due to personal concerns (Verbeke et al., 2015), food neophobia (Cox and Evans, 2008) and higher meat attachment (Bryant et al., 2019c), as these are some of the commonly observed reasons.

2.2.1.2 Food Neophobia and Meat Attachment

Food neophobia

Food neophobia is described as the fear of new foods, which is majorly seen among older consumers, while young and urban dwellers are usually less food neophobic (Flight et al., 2003). Food neophobia is driven by environmental factors or experiential factors (Galloway et al., 2003). Many researchers have studied food neophobia in the past few years (Cox and Evans, 2008; Demattè et al., 2014; Pliner and Hobden, 1992; Raudenbush et al., 1998; Tuorila et al., 1994), as it is observed in recent food technologies.

Effects of food neophobia on IVM

According to Wilks et al. (2019), “food neophobia is one of the strongest and most consistent predictors of people’s willingness to eat cultured meat (IVM) - and their perceptions of the benefits." Therefore, the higher the food neophobia, the lower will be the consumer acceptance towards IVM. Similarly, Bryant et al. (2019), also studied the effect of food neophobia on IVM purchase likelihood among the US, Indian and Chinese consumers. The results show that higher food neophobia leads to lower purchase intentions among US consumers. However, these findings are not true for Indian and Chinese consumers, since Chinese consumers had higher purchase intentions and lower food neophobia, whereas Indians had higher purchase likelihood despite higher food neophobia. Such findings are possibly due to the involvement of educated, high income and urban-dwelling consumers, who were already familiar with IVM.

Meat attachment

According to Graça, "Meat attachment refers to a positive bond towards meat consumption. It comprises of four dimensions: hedonism, affinity, entitlement and dependence" (Graça et al., 2015). Meat attachment is predominantly seen among the US consumers compared to others. It is mainly due to cultural differences in diets, since western diets mainly consist of 'meat' and vegetables, whereas this may not be the case among Indians or other consumer groups.

The author claims that consumers with higher meat attachment are less motivated to change their dietary habits (Graça et al., 2015). This is supported by findings from Bryant et al. (2019) study which shows that the US consumers with higher meat attachment have reduced purchase intentions towards IVM.

2.2.1.3 Personal concerns

2.2.1.3.1 (Un)naturalness

The perceived (un)naturalness is one of the critical factors for the acceptance of novel food products and food technologies (Roman et al., 2017). Therefore, a considerable amount of literature has been published on naturalness (Evans et al., 2010; Rozin, 2005; Rozin et al., 1999; Rozin et al., 2004). Research suggests that concerns about perceived unnaturalness are an in-built heuristic feature that indicates the quality of food (Rozin et al., 1999).

In addition, Rozin et al. (2005), states that unnaturalness concerns are likely due to the processes involved, rather than the content. Just like the unnaturalness concern, which arises from the processes involved in the production of IVM (Laestadius and Caldwell, 2015; Marcu et al., 2015; Verbeke et al., 2015; Welin, 2013; Wilks and

Phillips, 2017), due to this reason, some consumers refer IVM as “fake meat” (Bekker et al., 2017), “lab” and “test tube” (Laestadius, 2015a), “undesirable and problematic product” (Laestadius and Caldwell, 2015).

On the other hand, European consumers also termed IVM as critical such as “weird” (Verbeke et al., 2015), “ridiculous” (Verbeke et al., 2015) and “completely unnatural” (Verbeke et al., 2015), “playing God” (Marcu et al., 2015), “messing with nature” (Marcu et al., 2015). Furthermore, IVM was also linked with “in-vitro fertilisation” (Verbeke et al., 2015), “Frankenstein’s monster” and “Frankenfoods” (Marcu et al., 2015) and other future dystopian human societies as seen in science fiction movies (Marcu et al., 2015) due to IVMs unnatural nature.

Whereas, some consumers established the “natural vs artificial” polarities to distinguish between traditional meat and IVM, as consumers believe that anything natural as being good/healthy, and anything unnatural is terrible or carries risks. Similar findings were seen Seigriest et al. (2018) study. However, such disclination towards IVM is probably arising due to the comparison of IVM with other genetically modified foods, which also has unnaturalness concerns (Baron and Leshner, 2000; De Barcellos et al., 2010; Frewer et al., 2011; Marcu et al., 2015; Rozin et al., 1999; Scott et al., 2016; Siegrist et al., 2016).

On the other hand, Siegrist showed that concerns on unnaturalness evoke disgust and indirectly affect the perception of IVM (Siegrist et al., 2018). However, only one study has further investigated the effect of perceived unnaturalness on disgust Siegrist et al. (2018). The author in his work states that these concerns can be resolved by providing consumers with information on IVMs potential benefits, similarities between IVM and traditional meat (Siegrist et al., 2018) and by researchers exposing slaughterhouse practices (Dayal, 2005).

2.2.1.3.2 Disgust factor

Disgust is the most common initial reaction arising from IVM unnaturalness (Pluhar, 2010; Siegrist et al., 2018; Van der Weele & Driessen, 2013; Verbeke et al., 2015). Similar issues are observed with other genetically engineered novel foods (Frewer et al., 2011), which also suffer from reduced sensory properties and universal repugnance. According to some researchers, they say that disgust reactions are mere moral judgements and “reminders of livingness or animalness” (Martins and Pliner, 2005; Schnall et al., 2008). Whereas, Bryant et al. (2019), showed that disgust was a significant predictor of consumer acceptance of IVM, which indicates that higher the disgust, lower is the acceptance of IVM.

According to Hopkins, disgust reactions need to be further explored to analyse the reasons behind consumers, as it can help us identify it is caused by something immoral or if it is merely a neophobia (Hopkins and Dacey, 2008). Besides that, research shows that disgust is also linked to food neophobia as discussed in section 2.2.1.2.

On the other hand, some researchers argue that the disgust reactions towards IVM could be altered by providing more information to consumers (Siegrist et al., 2018), a detailed discussion on provision of information to manage personal concerns is under section 2.2.1.3.6

2.2.1.3.3 Palatability problems

Consumer concerns on sensory properties of IVM are observed in the literature (Hocquette et al., 2015; Verbeke et al., 2015). These palatability problems include issues such as poor taste, texture and appearance, are a result of technological setbacks of IVM, such as lack of iron, myoglobin, fat and other essential compounds that makes up meat. However, researchers are currently working on the setbacks by coculturing with fat cells to improve palatability problems. Nevertheless, as Post mentions, "creating or mimicking the natural flavour in IVM is a gigantic challenge because more than a thousand water soluble and fat derived components bring about the flavour of meat" (Post, 2012). A plausible solution to palatability problem is to mimics the sensory properties of traditional meat by coculturing with fat cells (Hoek et al., 2013).

The literature review shows that consumers believe that IVM is likely to have inferior taste, texture and appearance (Hocquette et al., 2015; O'Keefe et al., 2016; Slade, 2018; Tucker, 2014). About 29% (n=1001) of consumers had concerns with the taste (Grocer, 2017), about 38% (n=817) of consumers believe that IVM will not be tasty (Hocquette et al., 2015), possibly due to its unnaturalness. About 41% (n=533) of consumers think IVM would taste worst (Slade, 2018). Similar findings have been reported by Wilks et al. (2017) and Verbeke et al. (2015). Some consumers believe that IVM would be "soft" and "boring texture" (Bekker et al., 2017), unlike traditional meat. Similar findings were observed among NZ consumers due to unpalatable texture

(Tucker, 2014). Due to these reasons, some consumers wanted to compare IVM and traditional meat “side by side”, as consumers believe that “aesthetics of food is important as well” (O'Keefe et al., 2016).

The difference in taste perceptions could be linked to two reasons, firstly the idea of meat-eating (traditional meat) is considered as a pleasurable activity, but this may not be matched by IVM due to its nature (Hocquette et al., 2015) and stigmas associated. Secondly, concerns on taste and texture may be due to negative perceptions triggered by the unnaturalness of IVM.

2.2.1.3.4 Price

Literature has reported that there are speculations and concerns on IVMs pricing. Some consumers believe that IVM would be cheaper, while some consumers believe that IVM will be expensive than traditional meat (Bekker et al., 2017). The Literature shows that about 20% (n=673) of the US consumers are worried about the price of IVM (Wilks & Phillips, 2017). Whereas, 29% (n=1001) of European consumers have concerns about price (Grocer, 2017).

Consumers were reluctant to pay a premium price for IVM (Mancini and Antonioli, 2019). About 42.4% (n=180) of consumers from Belgium, Portugal and the UK would not pay extra (Verbeke et al., 2015) for IVM. Similarly, 26.7% (n=525) of Italian consumers were unwilling to pay a premium price for IVM (Mancini and Antonioli, 2019). Such results are likely due to poor purchase intentions, which are backed by consumer dietary preferences and their meat purchasing behaviour. In another study, only 43.9% of consumers from Belgium, Portugal and the UK were reported to show some willingness to pay a premium price for IVM, whereas 13.9% were surely willing to pay more (Verbeke et al., 2015b). However, the willingness to pay more increased from 13.9% to 35.8%, when consumers were informed about IVM perceived benefits. The willingness to pay a premium price may have been due to environmental values among consumers (Mancini and Antonioli, 2019), this is only a supposition.

General concerns on IVM manufacturing costs

Research highlights consumer concerns on the current manufacturing costs for IVM, as some consumers think that the “current price (3500$) of IVM would make it only available as a novel item to higher-income population (Laestadius and Caldwell, 2015; Verbeke et al., 2015). Whereas, the other consumers feared that IVM would be feed to the poor" (Bekker et al., 2017; Laestadius and Caldwell, 2015; Tucker, 2014; Wilks and Phillips, 2017). These differences in perceptions are due to several reasons, firstly, due to constant comparison between IVM and traditional meat, consumers believe that IVM is inferior to traditional meat, thus consumers expect IVM to be priced equivalent or cheaper than traditional meat. Secondly, because there are no IVM products available in the market to use a reference for comparing price. Thirdly, the media may have influenced these concerns by announcing the initial manufacturing costs of 350,000$ (Laestadius and Caldwell, 2015). However, these are common views held by the public towards a commercially unavailable product, hence these should be considered with care.

2.2.1.3.5 Perceived risks from the technology involved in IVM and food safety

2.2.1.3.5.1 Health risks

Research indicates that most consumers are worried about the perceived health risks of IVM, as about 4% (n=673) of US consumers fear about health risks from IVM consumption (Wilks and Phillips, 2017). Likewise, 37.5% (n=817) of European consumers think that IVM will be unhealthy (Hocquette et al., 2015). Similar findings have been observed in other quantitative studies in Europe (Verbeke et al., 2015b), the USA (Wilks and Phillips, 2017) and NZ consumers (Tucker, 2014). Thus, many consumers believe that is less healthy compared to traditional meat (Laestadius and Caldwell, 2015; Verbeke et al., 2015).

Besides, consumer worries are due to potential errors within IVM production (Verbeke et al., 2015). These concerns on healthiness are likely due to fear of human-made genetically modified foods, which have a higher likelihood for errors and mutations. Besides, techno-scepticism and neophobia also affects the perceptions of healthiness.

2.2.1.3.5.2 Safety concerns

Safety is one of the main concerns commonly observed in meat alternatives produced using GMOs (Genetically Modified Organisms) (Hocquette et al., 2005). Subsequently, concerns on health and food safety posed by IVM is also widely seen in research. Health concerns are based on perceived unnaturalness (Verbeke et al., 2015), whereas food safety concerns are based on scientific uncertainties (Siegrist and Sütterlin, 2017).

Research suggests that 56% (n=1001) of European consumers are worried about chemicals and ingredients in IVM, whereas 49% (n=1001) of consumers are concerned about long-term side effects, and 48% are anxious about unnaturalness (Grocer, 2017). On the other hand, some consumers are worried about the risk of cancer (Laestadius and Caldwell, 2015). While other consumers fear that these cells may undergo mutations and turn out to be cancerous. These concerns are likely due to the involvement of stem cells in IVM production, the nature of the product, the processing involved and due to the perceived unnaturalness, which triggers a safety concern among consumers. However, these concerns may not be valid as Food and Drug Administration (FDA) and United States Drug Administration (USDA) recently concluded cultured cells (IVM) and metabolites as generally recognised as safe (GRAS) (Watson, 2019)

On the contrary, only 3-4% (n=673) of US consumers felt that IVM had safety concerns (Wilks and Phillips, 2017). This is possibly due to lower fear towards safety, as the US extensively industrialised. Furthermore, such safety concerns are frequently discussed in focus groups or online commentary studies but not in consumer surveys. Such findings could be because of an unclear perception of safety among consumers.

2.2.1.3.6 Lack of familiarity

Most novel and unfamiliar foods are susceptible to dislike and repulsion, despite its potential benefits (Lupton and Turner, 2018). Thus, familiarity is essential for consumer acceptance because unfamiliarity and incomprehensibility lead to opposition. Similar views are observed in the case of IVM as well, since it is a novel technological development which is unfamiliar to the public.

Research suggests that 13% (n=180) of Belgium consumers (Verbeke et al., 2015b) and 14% (n=1296) of Netherlands consumers are familiar (Flycatcher, 2013) with IVM. These familiarity rates are likely due to increased media coverage, research and events mainly focused on the European countries. Furthermore, the familiarity among consumers may be due to the IVM burger unveiling event which took place in Europe.

On the other hand, about 57.3% (n=987) of US consumers, 35.5% (n=1019) of Chinese consumers and 25.5% (n=1024) of Indian consumers were not familiar with IVM (Bryant et al., 2019c). This is likely due to poor awareness, familiarity and media coverage in the Western countries (USA and Canada) and other Asian countries

(Thailand, Vietnam, and Malaysia). However, the familiarity readings from India and China may not be very accurate as the sample population was skewed with respondents who were already familiar with IVM.

Nevertheless, general lack of familiarity with IVM is due to several reasons, firstly due to the commercial unavailability of the product in the market, secondly due to lack of scientific knowledge about recent food technologies among consumers. Thirdly, due to ineffective marketing strategies used by companies, which may have been unsuccessful in reaching a wider public. Fourthly, due to poor personal interests on IVM among consumers. Lastly, due to the lack of media coverage on IVM technology and its perceived benefits. Furthermore, previous studies have reported that lack of familiarity leads to scientific distrust (Siegrist and Sütterlin, 2017), uncertainty and fear of adverse consequences (Verbeke et al., 2015b).

However, familiarity can be increased by the provision of relevant information. For example, some of the European Union institutions and governments have taken up PUST (Public Understanding of Science and Technology) method to engage, involve and familiarise public about recent scientific innovations to 'eliminate fears' among consumers. These initiatives can be implemented by other countries to create more awareness about IVM and eventually increase the familiarity and acceptance among consumers.

Furthermore, familiarity can be created in other alternative ways, such as either by providing consumers with relevant information or by increased media coverage on IVM. The former method has been carried out in previous studies, some researchers suggest that information provision lead to increased support (63%) (n=1296), acceptance and increased willingness to try (52%) (n=1296) (Flycatcher, 2013). Furthermore, informing consumers about IVMs environmental benefits led to an increased positive perception among consumers (Verbeke et al., 2015b). On the other hand, some researchers suggest that information provision influences the explicit attitude [2]of consumers towards IVM (Verbeke et al., 2015b). Whilst some researchers suggest that depending on the direction of the information provided, it can either lead to acceptance or rejection of IVM as a sustainable alternative (Bekker et al., 2017).

[2] Attitudes that are deliberately said and reported by an individual.

2.2.1.3.6.1 Lack of familiarity due to inefficient media coverage

Efficient media coverage is an important determinant of IVM acceptance among consumers, as the content and quality of the coverage will either result in acceptance or rejection. For example, if the media informed the consumers of general practices in livestock production, animal slaughter or meat processing, consumers' perceptions of the ‘real meat’ would change, and resultantly consumers would be more open to IVM. A classic example for this is the Netherlands, as intense media coverage on IVM development led to in increased acceptance among consumers prior to the IVM burger unveiling event (Driessen and Korthals, 2012). Similar findings have been observed among Italian consumers (Mancini and Antonioli, 2019).

But, research shows that if overemphasised by the media, it may result in scepticism and consumer resistance at the same time (Verbeke et al, 2007). Like the print media’s attempts to popularise IVM, which failed to impress the US and European consumers, as it involved detailed technical information on IVM which was uninterpretable by the public (Goodwin and Shoulders, 2013). Thus, the media plays an important role in shaping consumer opinions on novel agri-food technologies.

2.2.1.3.7 Socio-cultural beliefs

2.2.1.3.7.1 The cultural importance of meat

Most food choices are not only based on sensory properties, but also on culture and identity (Bisogni et al., 2002; Fischler, 1988; Mennell et al., 1992). Besides, it also depends on religion, region and personal preferences. Most culture-based diets such as the Western, the European, Middle-eastern, Oriental and Mediterranean diets are majorly meat-based, which typically consists of meat and vegetables/potatoes (Bove et al, 2003; J. L. Brown and Miller, 2002; Marshall and Anderson, 2002; Tucker, 2014). Meat consumption is a major part, as it considered as a pleasurable and luxury habit, (Polkinghorne et al., 2008), which involves psychological factors such as “cultural” and “lifestyle” (Font-i-Furnols and Guerrero, 2014). However, the factors mentioned above may not be applicable to IVM, as it considered as stigma and is socially unacceptable.

Furthermore, only two studies have explored the cross-cultural perceptions of IVM (Bekker et al., 2017; Bryant et al., 2019c), the findings from these studies point out the cultural differences and concerns faced by consumers. Some consumer groups were doubtful whether IVM is a type of meat or a meat replacement. Whereas, Chinese were more open with their intentions (Bekker et al., 2017) and exhibited higher acceptance (Bryant et al., 2019c) than other consumers. The acceptance from Chinese consumers could be due to their unconventional eating habits and openness to different kinds of meat, such as dog, snake, duck and pigeon (Newman, 2000).

On a different note, Bekker et al. (2017), points out that culture, religious approval and the society plays an important role in meat preferences since it affects the choice and edibility of meat, irrespective of their potential benefits. However, it is uncertain if such ideologies exist for IVM consumption.

2.2.1.3.7.2 Societal concerns

Research has demonstrated that consumers are worried about potential consequences of IVM, especially socio-cultural practices like barbeques (BBQs) and Sunday roast using IVM, as it may not match traditional meat BBQs and Sunday roast (Verbeke et al., 2015).

Besides, consumers are also worried about the adverse effects of IVM, such as the “loss of farming traditions and jobs”, “loss of culinary traditions, loss of cultural practices around meat consumption and rural livelihood” (Verbeke et al., 2015).

Furthermore, research showed that European consumers worry about IVMs negative impacts on traditional farmers (Bekker et al., 2017; Wilks and Phillips, 2017), as it could affect their livelihood. while some consumers believe that the innovation of IVM was driven by ‘vast profits, or fame’ (Laestadius and Caldwell, 2015). However, the concerns mentioned above are not seen in US consumers, possibly due to extensive industrialisation in the west compared to other countries.

2.2.1.3.8 Ethical concerns

Ethical concerns are one of the most discussed topics in IVM literature (Dilworth and McGregor, 2015; Hocquette, 2016; Hopkins and Dacey, 2008; Laestadius, 2015a; Miller, 2012; Pluhar, 2010; Stephens, 2013; Van der Weele and Driessen, 2013). Research showed that about 24% (n=673) of the consumers have ethical concerns about IVM (Wilks and Phillips, 2017) mainly due to its unnaturalness and due to perceived adverse consequences like human cannibalism, food inequality (Laestadius, 2015a; Laestadius and Caldwell, 2015), “loss of culinary traditions, rural livelihood, the preservation of livestock, open pace and diversity” (Verbeke et al., 2015). On the other hand, few consumers perceive IVM as the most ethical due to “zero environmental impacts and no ethical problems”(Verbeke et al., 2015b). Thus, there are both positive and negative perceptions on ethicality of IVM; these perceptions will be discussed in detail in the sections 2.2.1.3.8.1 onwards.

2.2.1.3.8.1 Concerns on animal welfare

Previous studies have reported ethical concerns on the way IVM works since it works on the principle of "unpleasant tissue culture practices" (Catts and Zurr, 2013b; Driessen and Korthals, 2012; McHugh, 2010; Miller, 2012), and usage of animal-derived cells and serums (FBS/ FCS) in IVM, which do not meet the objective of animal-free/cruelty-free nature of IVM. However, IVM proponents assert that animal-free media will be available shortly (Stephens, 2013), which can replace the animal-derived medium.

Furthermore, there are concerns about the extraction of cells from donor animals for IVM production, as some consumers believe that even "a single cell taken from a farm animal would be just as bad as having 40 billion animals raised and slaughtered each year" (Stephens, 2013). However, IVM proponents argue that biopsy under anaesthesia is ethically better than killing that many animals (Stephens, 2013).

On the other hand, consumers are also cynical about IVM's potential benefits on animal welfare, since consumers believe that there are other sustainable methods of livestock rearing, with improved animal welfare and reduced animal suffrage (Hocquette, 2016).

2.2.1.3.8.2 Concerns on environmental sustainability

Literature has reported that IVM has the potential to save the planet from global warming and environmental pollution (Hocquette, 2016) by reducing GHG emissions, land and water usage (Tuomisto and Roy, 2012). But, consumers are sceptical of IVMs role in reducing environmental effects (Hocquette et al., 2015; Hocquette et al., 2013), as they believe that IVM requires higher industrial energy compared to the energy demands in current livestock practices (Tuomisto and Teixeira de Mattos, 2011), thus questioning IVMs role in environmental sustainability.

2.2.1.3.8.3 Concerns on the technology involved

The literature have found that consumers have ethical concerns on the technologies involved in IVM (Laestadius, 2015a; Laestadius and Caldwell, 2015; Verbeke et al., 2015a), as consumers are dubious of combining technology and food. These concerns are likely due to food neophobia and due to IVMs artificial nature.

Furthermore, there are ethical concerns on the cell culture technique involved in IVM. A major concern is its ability to lead to human cannibalism or victimless cannibalism (FuturePundit., 2003; McIlroy, 2006; Peterson, 2006; Schneider, 2012), a way to culture human-animal meat. This is a concern to many consumers since both cannibalisms use cell culture techniques. Hence, consumers fear of IVM risks and adverse consequences.

On the other hand, many consumers believed that "IVM has failed to challenge the principle of animal meat consumption", as it did not result in the reduction of meat consumption levels (Laestadius, 2015a) since a majority of the consumers are still inclined towards traditional meat over IVM. Besides, some consumers believe that IVM may "reproduce the cultural desirability of meat" and in fact lead to increased levels of consumption (both traditional meat and IVM) (Cole and Morgan, 2013; Miller, 2012). This means that the negative repercussions caused by traditional meat industry will persist, despite the availability of environmentally sustainable meat alternatives.

Some consumers perceive IVM as a mere waste of resources mainly due to two reasons, firstly, because consumers believe that IVM is unrequired, as there are already other sustainable meat alternatives available in the market. Secondly, because consumers think that the money and time invested in IVM can be utilized better by investing in something more beneficial to society (Laestadius, 2015a). However, these opinions may be from individuals who are pro-traditional meat or have a higher attachment to traditional meat.

2.2.1.3.8.4 Concerns on fair trade

Conventionally, IVM technology does not belong to any individual or company but is produced by many start-ups in a laboratory setup, without the involvement of traditional farmers. Many consumers are worried that multinational businesses from the Western countries may get involved in IVM in later stages and eventually "consolidate their power in the food systems" and leave traditional farmers with no work. Thus, there are ethical concerns regarding fair trade towards farmers (Jean-François Hocquette, 2016) as it might affect their livelihoods.

2.2.1.3.8.5 Concerns on the unnaturalness of product

The literature highlights many ethical concerns on IVM, but objections like "IVM is an unethical development" (Laestadius, 2015a) stands out among many other protests. These concerns are mainly due to personal perceptions like "moving away from the natural would be unethical", which is why some consumers perceive IVM to be a negative development due to the unethical tampering with nature. However, such concerns are also observed with other novel technologies (Corner et al., 2013; Deckers, 2005; Frewer et al., 2011).

2.2.1.3.8.6 Concerns regarding animal extinction

Literature reports concerns regarding the ambiguous fate of farm animals on replacement of traditional meat with IVM. On this note, some consumers fear that promoting IVM may result in the extinction of farm animals, as they believe that if they are not consumed, or "they are no longer needed" for consumption (Laestadius, 2015a) then it would be extinct.

2.2.1.3.8.7 Concern regarding IVM leading to food inequality

Although IVM is considered as a solution for food security, many consumers fear that IVM will merely be a novelty product for affluent consumers (Cole and Morgan, 2013; Metcalf, 2013) and will be inaccessible to consumers from other income groups. While other consumers suspect that IVM would be potentially slipped/fed to the underprivileged consumers, especially to third world countries as a cheap source of protein (Verbeke et al., 2015). However, these claims and reactions are based on the hypothetical perception that IVM is an inferior product compared to traditional meat (Laestadius, 2015a).

However, despite the differences in ethical perception, some consumers still believe that IVM has numerous benefits towards the environment, human health and animal welfare compared to traditional meat. As a result, few consumers have shown acceptance towards IVM, which will be discussed in section 2.2.2.

2.2.2 Consumer Acceptance

Despite numerous concerns, some consumers continue to support the idea of IVM due to benefits towards the environment, human health and animal welfare. Generally, consumer acceptance of IVM mainly depends on three main factors, firstly on the socio-demographics. Secondly, on the consumers' general views on food technologies and lastly on their attitudes towards the environment and animal welfare.

2.2.2.1 Based on socio-demographics

Demographics is an important predictor of IVM acceptance, which mainly depends on gender, age, education, income, eating habits and political affiliation of potential consumers. These demographic parameters will be discussed in detail in the paragraphs 2.2.2.1.1 onwards.

2.2.2.1.1 Gender

Gender is one of the most significant predictors of consumer acceptance of IVM. Previous research shows that meat-eating is commonly seen as a masculine trait (Rothgerber, 2013), as men prefer “real” meals which consist of meat since it invokes a feeling that is central to their sense of self (Rothgerber, 2013; Slade, 2018). Such gender-based acceptance is seen in IVM as well.

Prior studies showed that men are more open to IVM compared to women (Mancini and Antonioli, 2019; Slade, 2018; Tucker, 2014; Wilks and Phillips, 2017). Such evident support is likely backed by western masculinity (Nath, 2011) and due to eagerness among male consumers to try newer technologies in general (Chau and Hui, 1998; Laukkanen and Pasanen, 2008).

Research has also found that generally women have negative attitudes towards meat-eating. Consequently, women are more inclined to either reducing meat or following vegetarianism/veganism (Rothgerber, 2013). Henceforth, vegetarianism/veganism is considered a feminine trait (Adams, 1990). On this note, previous studies have shown that women are very sceptical of trying novel food innovations, due to the fear of unknown risks and food safety threats (Bäckström et al., 2003). Similar findings are observed in IVM as well, as women have disclination to engage with IVM (Tucker, 2014). Conversely, Bryant et al. (2019) point out an unusually higher acceptance rate among Chinese women. However, these results could due to skewed sampling which consisted of young, educated and urban-dwelling women consumers, who were already familiar with IVM

2.2.2.1.2 Age

Age is another critical factor that determines the acceptance of IVM. Research shows that young consumers, particularly under 25 years are more receptive to IVM and are more likely to purchase IVM, compared to older consumers (Slade, 2018). This is because youth and education are the early adopter's traits for any new technology (Rogers, 2003).

On the other hand, research shows that elderly consumers' are less willing to try IVM (Mancini and Antonioli, 2019), it is because they are not open to newer experiences, and prefer not to deviate from their accustomed habits (McCrae et al., 1999) like traditional meat consumption.

2.2.2.1.3 Education

Some researchers have studied the effects of education/ qualification levels on IVM acceptance. The literature reports that participants with higher education have a higher willingness to try IVM, particularly the young (Under 25) and the educated (Kirshenbaum, 2018; Slade, 2018) compared with the less educated consumers. These findings are because educated consumers are likely to make rational decisions and not based on the naturalness of the product (Sinclair, 2014). On the contrary, Hocquette (2015) found that educated consumers believe that "artificial meat will not necessarily reduce animal requirements" or will not dramatically reduce the carbon footprint of traditional meat production. Thus, these consumers felt that IVM would not serve the purpose. Nevertheless, the reasons for contradictory findings is unclear, whether it is due to differences in the study design or due to cultural differences. However, such a result may be due to the involvement of meat industry professionals and scientists in the study.

2.2.2.1.4 Eating habits

Omnivores

Eating habits are another critical predictor of IVMs consumer acceptance. Previous research shows that individuals with higher meat consumption are less receptive to IVM (Tucker, 2014; Wilks and Phillips, 2017). These findings are likely due to higher meat attachment, food neophobia and usual reluctance to try new foods among consumers.

Few studies have shown that some meat-eating consumers consider IVM as a meat substituent for beef; consequently, such consumers have a higher inclination towards IVM. These findings are in line with, Mancini et al. (2019) study, which shows that about 57% (n=575) of meat-eating Italian consumers were willing to try IVM compared to other consumers with different eating habits. Likewise, even Chinese consumers with higher meat consumption rates exhibited higher purchase likelihood (Bryant et al., 2019c).

Pescatarian

Pescatarians believe that IVM would be healthy and tasty, indicating that pescatarians may have higher inclination towards IVM (Bryant et al., 2019c; Wilks and Phillips, 2017). However, Wilks et al. (2017) reported that consumers were most unlikely to eat fish if made as IVM.

Vegetarian and vegan

The perception of IVM by vegetarians and vegans are somewhat different, as research showed that vegetarians have a slight inclination towards IVM (Slade, 2018) due to its 'meat substituent' image. However, the rate of willingness to engage in IVM appeared to be lowest by both vegetarians and vegans (Wilks and Phillips, 2017).

2.2.2.1.5 Country-wise

The literature points out that US consumers have higher willingness to engage with IVM (Wilks and Phillips, 2017) and higher purchase likelihood (Bryant et al., 2019c). Thus, US consumers are more receptive to IVM, which is likely due to higher meat attachment observed in the west. Furthermore, as Laestadius (2015) stated, such acceptance could be due to the "already industrialized nature of US agricultural landscape and food system" compared to the Europe. However, research also reports US consumers' disgust towards IVM as discussed previously in 2.2.1.3.2 section.

Consumers from European countries such as Netherlands, Belgium, Italy, UK have shown acceptance towards IVM (Flycatcher, 2013; Verbeke et al., 2015), such findings are possibly due to personal interests in IVM and inclination towards vegetarianism and veganism among Europeans.

Eurobarometer (2005) further reported that 88% (n=12369) of Cyprian consumers to 23% (n=12369) of Bulgarian consumers expressed their unwillingness to engage with the technology, such reactions are due to the aftermaths of a prevalent ban on GMO foods in European countries (Laaninen, 2015).

Emerging markets such as India and China have shown higher acceptance towards IVM (Bryant et al., 2019c). This could due to higher meat attachment and lower food neophobia. Furthermore, such acceptance is also due to increased familiarity with IVM among consumers, which reduces the risk of immediate rejection. However, the information on acceptance by Indian and Chinese consumers should be considered with care, as it involved skewed sample (Bryant et al., 2019c). Similar findings have been observed in other Asian countries (YouGov, 2018), which could be due to lower meat attachment and higher receptiveness to novel food technologies. In contrast, New

Zealand consumers have shown minimal acceptance towards IVM (Tucker, 2014), such findings could be due to two reasons, firstly, because of high levels of meat consumption among New Zealanders. Secondly, it is due to the nature of the sample population, as Tucker et al. (2014) study mainly consisted of elderly and female consumers.

2.2.2.1.6 Political affiliation

IVM acceptance and traditional meat consumption has a strong connection with political affiliation. Previous studies showed that consumers with the right/conservative affiliation are often pro-traditional meat (Ruby, 2012). These consumers believe that IVM would have adverse effects on traditional farming (Wilks and Phillips, 2017), unlike the traditional meat industry.

Left/ liberal political have been found to have a higher acceptance of IVM, as liberals perceived IVM as a solution to the global warming issues. Besides, liberals believed that IVM is an ethical, natural and tasty alternative; subsequently, these consumers have a higher willingness to eat (Wilks and Phillips, 2017). Similar findings have been observed among the US and Indian consumers (Bryant et al., 2019c). The continuous support from the left /liberals was likely due to the progressive and moral thinking towards issues such as animal welfare, environmental impacts and food supply (Wilks and Phillips, 2017).

2.2.2.1.7 Income

Studies have shown that consumers with higher incomes are more receptive to IVM compared to lower-income groups (Bryant et al., 2019c; Wilks et al., 2019). However, the relation between higher income and consumer acceptance is unclear. It is possibly due to higher environmental awareness, analytical thinking in educated consumers and probably due to their purchase behaviour.

On the contrary, Tucker (2014) argued that neither higher nor lower-income, but consumer groups with mid-range income 49,000$ - 110,000$ NZD are more receptive to IVM. Such reactions could be due to higher-income consumers opting for superior quality cuts of traditional meat, while the lower-income groups opt for cheap supermarket meat, particularly in countries like New Zealand.

2.2.2.2 Consumer acceptance of IVM for other benefits

Besides sociodemographic factors, such as gender, age, education, eating habits, political affiliation and income, consumers would also be willing to try IVM due to its perceived benefits on the environment, animal welfare, food security and benefits.

2.2.2.2.1 Environmental reasons

Livestock rearing and traditional meat industry have a lot of negative repercussions such as global climate changes, global warming, greenhouse gas emissions, increased carbon footprint and increased water and land usage. Whereas, IVM does not have any known adverse effects, in fact, it requires 99% less land, 45% less energy, 96% fewer greenhouse gas emissions compared to traditional meat industry (Tuomisto and Teixeira de Mattos, 2011). Besides, consumers do realise that IVM is environmentally sustainable, as it reduces the emission of greenhouse gases (Bekker et al., 2017; Laestadius and Caldwell, 2015; Verbeke et al., 2015; Verbeke et al., 2015b; Wilks and Phillips, 2017). For these reasons, consumers have expressed their willingness to engage with IVM (Laestadius and Caldwell, 2015; Verbeke et al., 2015).

2.2.2.2.2 Animal welfare

Many researchers claim that IVM is beneficial for animal welfare as it prevents animal suffrage associated with the traditional meat industry, by replacing it with large scale production of IVM using animal cell culture (Post, 2012; Tuomisto and Roy, 2012) where no animals will be slaughtered for meat purposes. Not many consumers feared that IVM may result in the reduction of animals (Laestadius and Caldwell, 2015; Verbeke et al., 2015) but in fact many consumers believed that IVM will improve the animal welfare conditions (Wilks and Phillips, 2017). Thus, consumers' acceptance towards IVM is mainly due to its potential benefits on animal welfare and animal ethics (O'Keefe et al., 2016; Tucker, 2014).

2.2.2.2.3 Food security

The United Nations stated that "about 815 million hungry people must be nourished today, and an additional 2 billion people are expected to require nourishment by 2050" (United Nations, 2015). However, this may not be possible with the current traditional meat industry practices as it is obsolete and has a lot of adverse effects, so global hunger has to be met by sustainable meat alternatives like IVM since it can potentially solve global meat demand issues and successfully overcome the limitations of the traditional meat industry (Tuomisto and Roy, 2012).

There are reports which show that consumers worry about food inequality, and that IVM will be used feed the underprivileged consumers (Laestadius, 2015a; Tucker, 2014; Verbeke et al., 2015). However, these assumptions on food inequality are imaginary, and are mostly towards a hypothetical product which is currently commercially unavailable.

2.2.2.2.4 Health benefits

Despite the initial scepticism about health and nutritional impacts of IVM, some consumers still believed that IVM has potential health and safety benefits such as higher safety, higher quality standards, and reduced risk of bovine diseases and zoonotic infections such as *Escherichia coli* and *Salmonella* infections, which are commonly found with traditional meat consumption (Bekker et al., 2017; O'Keefe et al., 2016; Verbeke et al., 2015; Wilks and Phillips, 2017).

Consumers believed that IVM was healthy because it had minimal fat content (Bekker et al., 2017; Laestadius and Caldwell, 2015; Verbeke et al., 2015), unlike traditional fatty meat, which was likely to cause cardiovascular diseases. On this note, the majority of consumers felt that IVM was entirely safe (Verbeke et al., 2015b). Likewise about 28.6% of consumers in Europe perceived IVM to be healthy (Hocquette et al., 2015). This might be due to the fact that there are no studies on the adverse health implications of IVM, unlike traditional meat, which has been reported to be related to colon disorders with excessive and prolonged consumption of meat (Bouvard et al., 2015; Larsson et al., 2005; McAfee et al., 2010).

2.2.3 Consumer Uncertainty about IVM

2.2.3.1 Scientific uncertainties of IVM

2.2.3.1.1 Controllability

Consumers are wary of the process controllability and worry about "mutations", bacterial contamination, risk management and potential violation in the production process (Verbeke et al., 2015). However, such confusions are mainly due to general scepticism towards novel food innovations and lack of familiarity among potential consumers. These concerns can be resolved as technology advances and become familiar with consumers. Furthermore, these concerns will decline once the public is aware of the food safety regulations put forth by prospective food companies.

2.2.3.1.2 *Feasibility*

Consumer concerns regarding the unfeasibility of IVM (O'Keefe et al., 2016; Verbeke et al., 2015; Wilks and Phillips, 2017) is evident in literature. There are concerns on the feasibility of making IVM affordable, IVM as an animal-free meat and IVM as a technology. Many consumers are worried about the IVM being affordable, as it perceived to be an expensive product due to its high manufacturing costs.

Some opponents argue that it is unfeasible to call IVM as an 'animal-free' meat since it includes foetal bovine serum (FBS), an animal-derived serum (Laestadius and Caldwell, 2015). Some consumers are unsure about the principle behind IVM technology and sceptical of IVM feasibility that can result in "massive production from a single cell". Doubts and uncertainties regarding IVM feasibility among consumers could be a result of lack of familiarity and relevant information provision about IVM technology. However, these concerns can be reduced as IVM gains popularity in future.

2.2.3.1.3 *Regulatory efficiency*

Due to the commercial unavailability of IVM products, there is no regulatory framework governing IVM until recently. For this reason, many consumers are worried about regulatory issues such as food quality (O'Keefe et al., 2016). As a plausible solution to these concerns, consumers demand health and safety checks and quality control checks of IVM before the product is available commercially.

Concerns regarding IVM food safety tests, marketing strategy and labelling of the products were raised by consumers to help with framing stringent regulations (Verbeke et al., 2015). Similar reactions were observed in other studies, where consumers believed that an efficient regulatory system was essential for consumers to build their trust with IVM (Laestadius and Caldwell, 2015). However, In November 2018, FDA and USDA along with the Good Food Institute issued a joint statement on regulations towards IVM (Ball, 2019). This joint agreement could potentially resolve all the trust issues faced by consumers and help establish trust in the future.

2.2.3.2 *Distrust in technology*

According to Bearth, "Trust is especially important in the absence of the possibility to judge the risk by oneself, which frequently is the case with new or complex food technologies" (Bearth et al., 2014). In the case of IVM, trust is implausible at this stage of development as distrust is commonly observed when consumers reject IVM technology.

Consumers concerns about IVM being introduced in the markets without our knowledge (Laestadius, 2015a) indicated that people do not trust corporate food manufacturing companies. They believed that food companies are more concerned about making money with less concern about consumers' welfare.

Distrust towards IVM can be attributed to consumers' lack of scientific knowledge, lack of trust in technology and the product, and lastly lack of trust on researchers involved in IVM production. Furthermore, distrust in IVM also arises due to the unnaturalness of the product. However, it can be resolved by improving familiarity among consumers, and by creating an efficient regulatory system in the future (Laestadius and Caldwell, 2015).

2.3 GENERAL BACKGROUND OF MUSCLE FORMATION

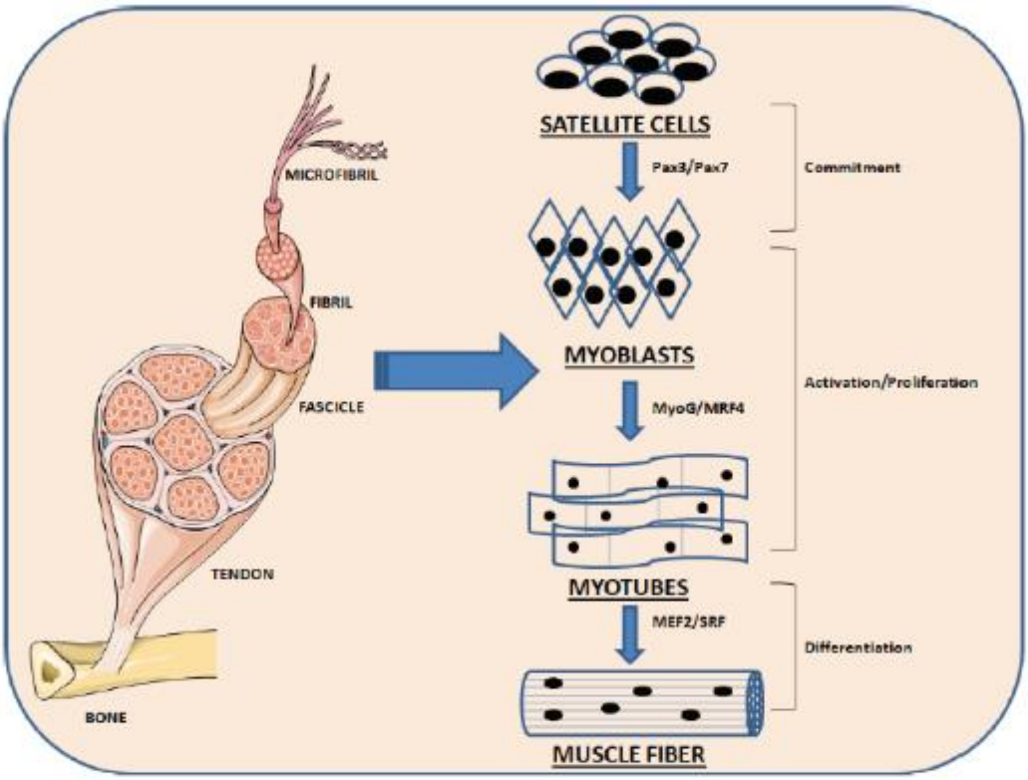

Figure 2-2 Schematic representation of muscle formation using skeletal muscle satellite cells, which undergoes proliferation and differentiation to produce a muscle fibre (Ultimo et al., 2018).

In-vitro meat (IVM) works on the concept of myogenesis, a process of muscle formation. The formation of muscular tissue occurs during embryonic development, by multipotent myoblasts cells of mesodermal origin. These myoblast cells undergo embryonic fusion, proliferation and differentiation (Langelaan et al., 2010), to form myotubes, which gradually combine to forms muscle fibres as seen in Figure 2-2. The process of skeletal muscle formation is analogous to the production of IVM because a piece of meat is merely a mass of edible muscle fibres. For further information on the process of muscle formation, the article by (Buckingham et al., 2003) is recommended as this is beyond the scope of my research.

2.4 IVM HISTORY AND DEVELOPMENT

IVM is also known as ‘cultured meat’, ‘lab-grown meat’ and ‘clean meat’ (The Good Food Institute 2017, 2018). It has also been referred to as ‘animal-free meat’, ‘slaughter-free meat’, ‘vat meat’ ‘synthetic meat’, ‘artificial meat’, ‘shmeat’, ‘frankenmeat’ and ‘test-tube meat’. The Food and Drug Administration (FDA, USA) United States Drug Administration (USDA, USA) and other in-vitro meat companies have jointly termed IVM as ‘cell-based meat’ (Watson, 2018a).

The concept of IVM has been prevalent since long as Churchill predicted the future and stated that, "fifty years hence, we shall escape the absurdity of growing a whole chicken in order to eat the breast or wing, by growing these parts separately under a suitable medium" (Churchill, 1932). On the other hand, research on in-vitro cultivation of muscle fibres began as early as 1971 when a researcher cultured immature aortal cells from guinea pig to obtain myofibrils on eight weeks of culturing (Ross, 1971). A few years later, another researcher cultured goldfish cells, which eventually developed into fish fillets (Benjaminson et al., 2002). During this period, a Dutch researcher turned entrepreneur, Willem Van Eelen received a patent for producing edible meat using collagen and muscle cells without killing animals (W. F. Van Eelen, 2007). Additionally, People for the Ethical Treatment of Animals (PETA), a US-based non-profit organisation exhibited their support towards IVM by announcing a prize money of $1 million-dollar to those who could cultivate lab-grown meat/in-vitro meat (IVM) using chicken cells (Newkirk, 2008). Besides that, the National Aeronautics and Space Administration (NASA) supported the idea of IVM since it can be used as a long-term food product for space missions (Bartholet, 2011). Several years later, IVM regained popularity as Dr Mark Post in 2013 created a beef burger patty using cow cells in a live event in London (BBC, 2013). Following this event, cell-based meat/ IVM technology has created much attention in the past few years and is one of the most researched current topics in the food industry.

Currently, IVM /cell-based meat research is supported by non-profit organisation such as The Good Food Institute (GFI) and start-up companies such as New Harvest, CUBIQ Foods, Mosa Meat, and Memphis Meats and JUST Inc. Furthermore, business tycoons such as Bill Gates, Sergey Brin and Richard Branson have invested in cell-based meat research and IVM start-up companies (Guedim, 2019), Henceforth, the number of investors in IVM and sustainable foods have increased exponentially. These investments have had a significant impact on the production of IVM as the costs for an IVM burger patty is now about $2400, which was $330,000 previously (Szondy, 2013). However, IVM proponents hope that the price will be as low as $5 by 2021 (Nelson, 2018).

The IVM industry is ever-increasing, and many start-up companies such as Memphis meat, Meatable, Mosa meat, Higher steaks, Finless Foods and JUST, Inc., in the US and European countries now produce cell-based meats. Besides, even southeast Asian countries have extended their support towards IVM with China recently signing a $300 million-dollar deal to import slaughter-free meat from an Israel firm (R. Roberts, 2017). In addition, Japan struck a deal with JUST Inc., to deliver cell-based Wagyu beef in the market (Watson, 2018b). According to industry experts, IVM will be commercially available in about five years due to huge technological advancement.

Negative repercussions of traditional meat industry and the need for meat alternatives

The Food and Agriculture Organisation (FAO) predicted that the rate of meat consumption will increase by two thirds, by about 73% in 2050 (Gerber et al., 2013). Such a tendency is expected to last for nearly four decades (Godfray et al., 2010). In other words, an increase in meat consumption levels will result in increased demand for meat products.

Increased meat production will result in increased adverse effects such as global climate changes, global warming, greenhouse gas emissions (GEG), increased carbon footprint and animal suffering. Besides, traditional meat production also causes water pollution due to the increased use of fertilisers and pesticides (Walker et al., 2005). According to the United Nations (UN) and FAO, about 30% land usage, 6% of freshwater usage and 14.5% of greenhouse gas emissions (Figure 2-3) are linked to the livestock meat industry (Edenhofer, 2015; FAO, 2006). Moreover, New Zealand alone emits 49.2% of its total GHG emissions from the agricultural sector (Ministry of the Environment, 2018a), which includes both the livestock and meat industry.

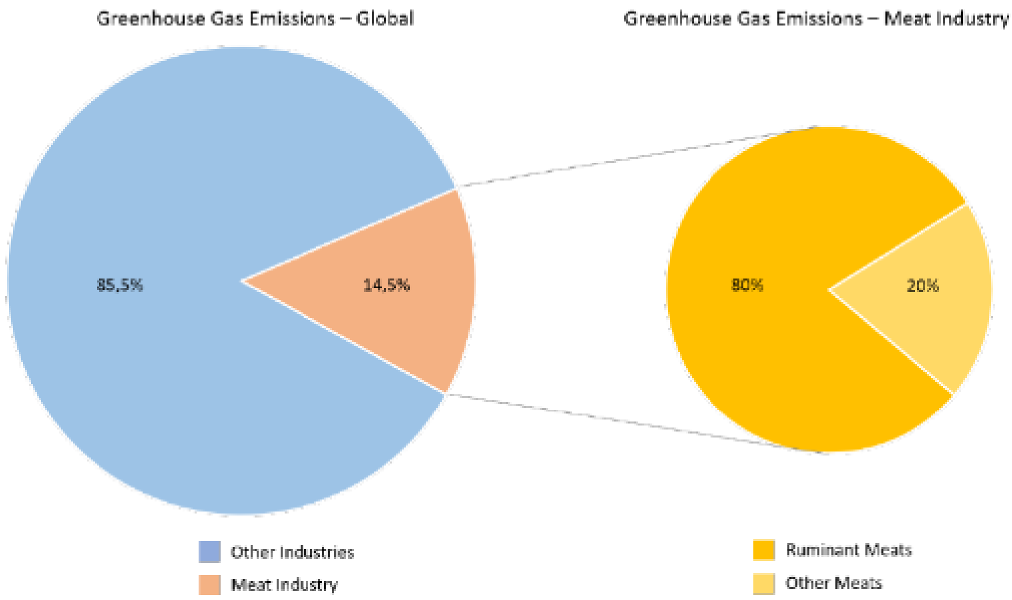

Figure 2-3 Greenhouse gas emission by the meat industry (Beaudoin et al., 2018; Clark and Tilman, 2017; Edenhofer, 2015; FAO, 2014)

For all the reasons mentioned above, there is a dire need across the world for sustainable meat alternatives such as IVM, to meet the global demand for meat. According to few researchers, IVM takes up 99% less land, 45% less energy, 96% fewer greenhouse gases emissions compared to the traditional meat industry (Tuomisto et al., 2014; Tuomisto and Roy, 2012; Tuomisto and Teixeira de Mattos, 2011). The greenhouse gases (GHG) emissions of IVM are as low as traditional pork and poultry industries (Mattick et al., 2015; Tuomisto et al., 2014). Furthermore, IVM is unlikely to cause any adverse environmental impacts; in fact it promotes the reversal of climate changes (Huiling, 2017).

IVM has other potential benefits (Bhat et al., 2015) such as health due to its production method. It has minimal fat content which reduces the risk of cardiovascular disease. Besides, it has lower food safety risk compared to traditional meat as it is produced in a sterile and controlled environment (McHugh, 2010), unlike the traditional meat which is susceptible to pathogenic contaminations. Furthermore, IVM is free of antibiotics, unlike traditional meat that overtly uses antibiotics (Donovan, 2015; Wang et al., 2017), which can result in antibiotic resistance among humans (Ghorbani et al., 2016; Sutherland, 2015). Apart from that, IVM is also beneficial for animal welfare as it does not involve breeding, suffrage and slaughtering of animals for meat purposes. Furthermore, IVM can be produced in large amounts at a lower price than traditional meat (Bhat et al., 2015) if technological advancements are made in the future. Henceforth, for all these reasons, IVM has gained popularity as the most sustainable, environment-friendly and animal-friendly meat alternative.

2.5 IVM PRODUCTION

In the last few years, many researchers have worked relentlessly to produce IVM efficiently. Previously muscles were grown in a petri dish (Benjaminson et al., 2002; Catts and Zurr, 2002a), while recently others have incorporated various culture conditions, bioreactors and various technologies such as scaffold method (Catts and Zurr, 2002a; Van Eelen et al., 1999), self-organising method (Benjaminson et al., 2002), micropatterning (Acevedo et al., 2018). Furthermore, other technologies such as organ printing, biophotonics, nanotechnology and 3D printing are currently being considered for IVM Production (Bhat and Bhat, 2011).

Currently, IVM is produced in a bioreactor where the extraction, cultivation, and harvest of cells of interest is carried out for IVM production (Figure2-4). The cells are later subjected to cell proliferation and differentiation stages in a bioreactor where muscle cells grow into muscle fibres. The desired cells are subjected to stimulations under optimum culture conditions, which facilitates cell growth. In most cases, the cells are grown on three-dimensional scaffolds as it provides highly structured meats like steaks, whereas in some cases, the cells can self-organise to form muscle fibres. However, self-organising technique does not offer structured meats but are suitable for processed meats like sausage, mince and patties.

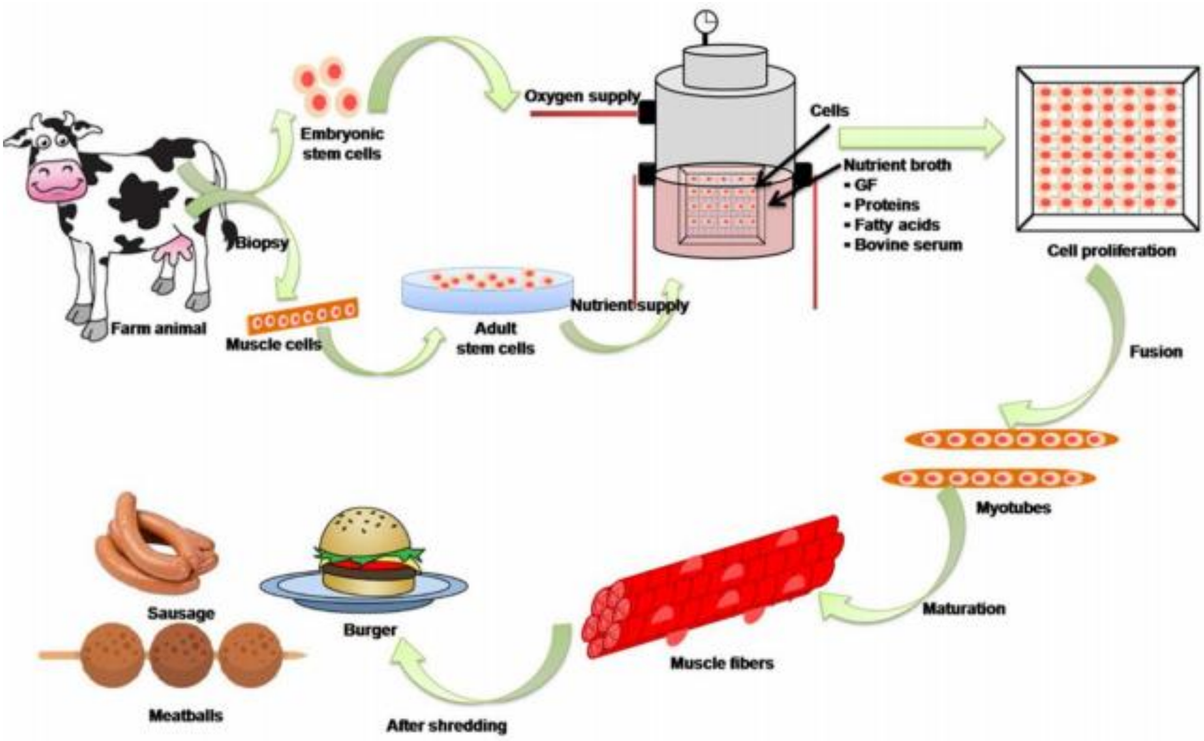

Figure 2-4 Schematic representation of IVM production (Gaydhane et al, 2018)

A brief account on the cells, culture condition, stimulation of cell and other information on IVM production are listed in sections 2.5.1 onwards. In addition, Table 2-1 summarizes general findings on conditions used for IVM production.

2.5.1 Cells and culture conditions

2.5.1.1 Cells

There are various cell sources for IVM production, and these include adult stem cells (ASC), tissue-specific stem cells, mesenchymal stem cells (MSC) and induced pluripotent stem cells (iPSCs). The most widely used cells are ESC and myosatellite cells (Edelman, McFarland, Mironov, and Matheny, 2005; W. Van Eelen et al., 1999).

Table 2-1 General findings on cell sources and culture conditions for IVM production.

Method	Cells	Bioreactor	Culture medium	Presence of growth factor/antibiotics	Culture conditions	Reference
Explant/ BAMS[3]/co-culture using perforated stainless-steel disks or cellulose acetate	Goldfish derived crude cell mixture and ATCC brown bullhead fibroblasts	N/A	1. Minimal essential medium (MEM)[4] With Hanks salts[5] and Earle's salts[6] with foetal bovine serum (FBS). 2. 10% FBS[7] in 90% MEM in Hanks' salts. 3. Collagenase + incomplete MEM (minus FBS) Hanks' salts. 4. FBS substituted with dried Shiitake or Maitake mushrooms and fish meal extracts.	No antibiotics	Temperature - 23°C pH- 7.2	(Benjamin son et al., 2002)
Scaffold - P(HEMA)[8], PGA[9],	Mc Coy Cell line – mouse endothelial cells	Rotating bioreactor-	Nutrient media	Growth factors and/or cytokines[12]	Cell cultured for 7-21 days in 37°C / 5% CO_2 incubator	(Catts and Zurr, 2002a)

[3] BAMS – Bioartificial Muscle System
[4] MEM – is a synthetic cell culture used to maintain cells in a tissue culture.
[5] Hanks salt - salt rich in bicarbonate ions, which are used as a buffer system in cell culture media and aid in maintaining the optimum physiological pH (roughly 7.0–7.4) for cellular growth.
[6] Earle's salt- a type of salt solution used for short–term maintenance of cells in a CO_2 environment.
[7] FBS – Foetal Bovine Serum
[8] PHEMA – Polyhydroxyethylmethacrylate
[9] PGA – Polyglycolide or poly glycolic acid
[12] Cytokines are groups of proteins, peptides or glycoproteins that are secreted by specific cells of the immune system

Method	Cells	Bioreactor	Culture medium	Presence of growth factor/antibiotics	Culture conditions	Reference
PLGA[10], PHB)[11]		Synthecon RCCSID4				
Scaffold- Sodium alginate and fish gelatin type-1 with gelling agents such as agarose and glycerol	Myoblast cell line C2C12	N/A	**Proliferation medium**: DMEM[13] media with 10% FBS + 2 mM L-glutamine, **Differentiation medium:** DMEM + 2% Horse serum	Antibiotics-penicillin and streptomycin	Cell cultured 37 °C and 5% CO_2 in a humidified atmosphere	(Acevedo et al., 2018)
Scaffold - alginate	C2C12 cells		1. **C2C12 Growth medium:** DMEM high glucose – 4.5g/l with sodium pyruvate without L- glutamine and 10% FBS. 2. **C2C12 differentiation medium:** same as above but FBS replaced by Horse serum. 3. PBS	1% of L-glutamine and 1% penicillin	Cell cultured in T-25 flasks at 310K with 5% CO_2 and 96% humidity. pH –7 to 7.3 At room temperature	(Schuster et al., 2017)
Self-assembled tissues on 3D	Murine C2C12 myoblasts, human dermal fibroblasts, or	N/A	**Growth media (GM)** consisting of DMEM with 10% FBS.	**GH**: 1 ng/mL of TGF -β1 (Transforming growth factor beta-1)	Cultured for 7 days with media replenishment every 2-3 days	(Krieger et al., 2018)

10 PLGA – Poly(lacto-co-glycolic acid)
11 PHB - Polyhydroxybutyrate
13 DMEM- Dulbecco's modified Eagle medium, a basal medium used for supporting the growth of cells.

Method	Cells	Bioreactor	Culture medium	Presence of growth factor/antibiotics	Culture conditions	Reference
agarose-PDMS[14] scaffolds	TGF-1-differentiated myofibroblasts		**Differentiation medium**: DMEM, 2% horse serum	**Antibiotics**: Penicillin and streptomycin		
Scaffold	Muscle cell	Stirred tank bioreactor	Cyanobacteria hydrolysate	Growth factors and vitamins	Cell cultured for 60 days at 37 ℃, at 100 rotations rpm aeration 0.05vvm[15]	(Tuomisto and Teixeira de Mattos, 2011)
Scaffold - collagen spores	C2C12 cells of permanent myogenic cell line	N/A	**Basal medium:** Dulbecco's modified Eagle medium (DMEM) **For expansion**: above media + 10% foetal calf serum (FCS). **For differentiation:** fusion medium (FM)containing 2% FCS, 1% Insulin-Transferrin-Selenium-A, 670 µg/l sodium selenite, 11 g/l sodium pyruvate, 1 g/l insulin and 550 mg/l transferrin	Antibiotics, Penicillin and streptomycin sulphate.	Cultured on collagen spores in a tissue culture flask. Incubated in a humid chamber at 37℃	(Kroehne et al., 2008)

[14] PDMS - Polydimethylsiloxane
[15] Vvm – volume of air per unit of medium per unit of time.

Method	Cells	Bioreactor	Culture medium	Presence of growth factor/antibiotics	Culture conditions	Reference
Scaffold – collagen (surgisis)[16]	C2C12 myoblast cells Human Umbilical Vein Endothelial	N/A	1.DMEM supplemented with 20% FBS, and 2.5% HEPES[17] buffer 2.Endothelial cell medium 3.DMEM supplemented with 10% FBS and 1% nonessential amino acids	VEGF (vascular endothelial growth factor) and FGF2 (fibroblast growth factor)	Constructs were placed in Krebs Henseleit[18] solution with 95% O_2, 5% CO_2 gas mixture at 25℃	(Koffler et al., 2011)
Scaffolds – Polydimethylsiloxane (PDMS)	Murine C2C12 myoblast cells	N/A	**-Growth medium**: containing DMEM + 10% foetal bovine serum (FBS) Matrigel was added for faster cell spreading,	1%-penicillin and streptomycin	N/A	(Bian and Bursac, 2009)

16 Surgisis – a bio-mesh with high collagen content used in biomedical processes.
17 HEPES – (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)
18 Krebs Henseleit solution is a nutrient rich solution (with sodium, chloride, calcium, magnesium sulfate, bicarbonate, phosphate, glucose, albumin and tromethamine) which is used in ex vivo studies.

2.5.1.1.1 *Myosatellite cells*

Satellite cells are the most preferred cell sources for the production of skeletal muscle tissue / IVM. Satellite cells are also known as myoblast cells / myosatellite cells or muscle stem cells, which belong to the adult stem cells (ASC) category. It exhibits multipotency and is analogous to embryonic myoblast cells (Kuang and Rudnicki, 2008). These are mononucleated adult stem cells (ASC) situated at the periphery of skeletal muscle myofiber. When the myofibers are damaged in an injury, the satellite cells undergo cell cycle to produce myoblasts which ceases cell cycle to form myofibers. These myofibers eventually form myofibrils and muscle tissues at later stages. Similar effects will be observed if these cells are used in skeletal muscle/IVM production; henceforth satellite cells are the most preferred cell source.

Furthermore, the literature points out that satellite cells/myoblast cells/adult stem cells (ASCs) are the most suitable type of cells due to their high regeneration power (Mauro, 1961; Post, 2012), its ability to replicate myogenesis (Benjaminson et al., 2002; Le Grand and Rudnicki, 2007) and its capacity to produce mature cells with specialised morphologies and functions, unlike the embryonic stem cells (ESCs). Hence, these cells are widely used in IVM research (Verbruggen et al., 2018), especially the C2C12 myoblast cells (Acevedo et al., 2018; Bian and Bursac, 2009; Ikeda and Takeuchi, 2019; Koffler et al., 2011; Schuster et al., 2017; Seo et al., 2019), and in bio-artificial muscle production (BAMS) (Dennis and Kosnik, 2000; Vandenburgh, et al., 1999). Moreover, ASCs such as epithelial cells have been used previously in the in-vitro muscle production system (IMPS) (J. Williams, 2012), but these cells require stimulation to form myoblasts, which may result in faulty myogenesis (Bach et al., 2003). Besides that, myosatellite / myoblast / ASC's have a few drawbacks such as limited proliferation rate and susceptibility to turning cancerous, if cultured for extended periods.

2.5.1.1.2 Embryonic stem cells (ESC)

Stem cells that are derived from the embryo are known as embryonic stem cells (ESC) (Haagsman et al.,, 2009), which are either extracted from bovine or porcine. These are preferred cell sources for IVM production (Catts and Zurr, 2002a) due to their pluripotency and unlimited self-renewal capacity. For this reason, some researchers claim that IVM produced using ESC could provide sufficient meat to feed global hunger (Bhat and Bhat, 2011). Thus, for the above reasons, ESC is considered as a cell source for IVM production.

There is currently no availability of bovine, ovine or porcine-derived ESC, and only murine cell lineages are available, which requires to be differentiated into myogenic progenitor cells (MPCs), before muscle fibres can be formed. Besides that, other concerns of ESC in IVM production are its requirements of cell differentiation in order to produce myoblasts (Datar and Betti, 2010; Langelaan et al., 2010; Mattick and Allenby, 2012). These stimulated cells are susceptible to loss of their existing proliferative characteristics at any later stages, despite culturing (Bhat and Bhat, 2011). In addition, there is difficulty in maintaining undifferentiated embryonic stem cells (Langelaan et al., 2010; Telugu et al., 2010), as opposed to other cell sources for IVM production.

2.5.1.1.3 Induced pluripotent stem cells (iPSC's)

Induced pluripotent stem cells are differentiated cells, which are already transfected to induce pluripotency in cells. These iPSC's can be used as a cell source for IVM production (Krieger et al., 2018; Roberts et al., 2015) due to their myogenic differentiation capacity and injury repair mechanism (Mizuno et al., 2010). However, there is no scientific evidence regarding their usage in IVM production. Only one study used fibroblast cell co-cultured with goldfish explant in the production of a bio-artificial muscle system (BAMs) (Benjaminson et al., 2002).

On a different note, iPSC's are often co-cultured with fat cells such as adipose tissue-derived stem cell (ADC) to improve the texture, flavour and tenderness of IVM by increasing the amount of intramuscular fat (Hocquette et al., 2010). These co-culturing techniques have been observed in other studies (Dodson et al., 2013; Frerich, et al., 2012; Seo et al., 2019; Verseijden et al., 2012).

2.5.1.1.4 Dedifferentiated cells

Dedifferentiated cells are the cells that have been reversed from terminally differentiated cells into multipotent cells, such as mature adipocytes. These cells on dedifferentiation gives rise to multipotent preadipocyte cell line known as dedifferentiated fat cells (DFAT) (Matsumoto et al., 2008). In addition, these cells produce skeletal myocytes (muscle cells) when transdifferentiated (Kazama et al., 2008). These cell properties make the cells suitable for use as a cell source for IVM production. However, some researchers argue that terminally differentiated cells properties of transdifferentiation, dedifferentiation and multipotency, maybe an unusual feature exhibited by cell-like substances, rather than the cell (Kazama et al., 2008; Matsumoto et al., 2008; Seo et al., 2019).

Despite the variety of cell sources available, IVM production is still a challenging task due to chances of cell death, but some studies suggest that death can be prevented either by using immortal cells or by immortalisation of cell lineage. Furthermore, another challenge is to create an environment (*in vitro*) that mimics *in vivo* conditions for the optimum growth of cells. Detailed information on culture conditions is covered in section 2.5.1.2.

2.5.1.2 Culture conditions

Typically, culture conditions include factors such as culture media, serums, growth hormones and parameters such as pH, temperature, oxygen potential, pressure and mechanical/electromagnetic/gravitational simulations for cells to produce IVM. However, defining a range, as well as optimising and stabilising the above parameters, is a challenging task, during large scale IVM production. Detailed information on the culture conditions is described below.

2.5.1.2.1 Culture medium

The culture medium plays a vital role in IVM production since it serves as a nutrient source for the growth of cells. The culture medium must be simple, edible optimal, affordable and readily absorbable, as it is used in considerable amounts.

Traditionally, natural medium such as blood plasma was used for animal cell culture (Burrows, 1910), but currently, researchers work with inexpensive serum-free medium such as Dulbecco's Modified Eagle Medium (DMEM) (Acevedo et al., 2018; Bian and Bursac, 2009; Koffler et al., 2011; Kroehne et al., 2008; Levenberg et al., 2005) and Ultroser G, which has all the essential nutrients, growth factors, adhesion factors and binding proteins (Duque et al., 2003). However, the disadvantage of animal-friendly and the serum-free medium is the cost involved. Nevertheless, few researchers suggest that culture medium with both plant-based extracts and partially purified growth factors are economical and beneficial for the growth of cells (Haagsman et al., 2009). Besides, the requirement for culture medium changes depending on cell growth /stages of development, such as differentiation and proliferation media used. Thus, there is a need for a continuous supply of edible and animal-friendly medium, which facilitates the growth of cells.

2.5.1.2.2 Serum

Traditionally, animal-based serums such as foetal bovine serum (FBS) (Acevedo et al., 2018; Bian and Bursac, 2009; Jossen et al., 2014; Koffler et al., 2011), foetal calf serum (FCS) (Jossen et al., 2014; Kroehne et al., 2008), fishmeal extract and horse serums were used. However, these serums are neither ethical nor economical to use in the long run. Furthermore, animal-based serums have a high risk of carrying pathogens (Coecke et al., 2005). Researchers are now working with animal-free serum such as algal (cyanobacteria) or fungal/mushroom based (Shitake and Maitake) (Benjaminson et al., 2002), which makes it animal-friendly in nature. Interestingly, these serum-free medium have higher growth rates compared to FBS (Benjaminson et al., 2002), due to sphingosine 1-phosphate and amino-acids in mushrooms (Datar and Betti, 2010; Edelman et al., 2005).

2.5.1.2.3 Growth factors

Growth factors are essential components for the production of IVM (Bhat and Bhat, 2011), but the formulation and optimisation of growth factors is a challenging task (Datar and Betti, 2010), as it is dependent on the type of cells used. The commonly used growth factors for IVM production include Transforming Growth Factor-β (TGF-β), Fibroblast Growth Factors (FGFs), Insulin-like Growth Factors (IGF) VEGF (vascular endothelial growth factor) and FGF2 (fibroblast growth factor) (Koffler et al., 2011). However, TGF- β decreases the myoblast recruitment and differentiation (Goetsch et al., 2003), whereas FGFs are more stimulatory in nature compared to TGF – β. FGFs can enhance the rate of myoblast proliferation and prevent differentiation (Hannon et al., 1996). Similar to FGFs, a splice variant of IGF-1, called mechano growth factor, increase proliferation of myoblasts (Ates et al., 2007) and differentiation in C2C12 myoblasts (Florini et al., 1996).

2.5.2 Processes involved in IVM production

2.5.2.1 Self-organising technique

The self-organising technique is a scaffoldless tissue engineering technique, which allows cells/tissues to grow freely using external forces such as physical manipulation or thermal input. Additionally, self-organisation works on the principle of tissue fusion as seen in Figure 2-5. Tissue fusion is a process in the developmental biology stage, where two or more identical tissues meet and fuse to form a continuous structure (Pérez-Pomares and Foty, 2006; Ray and Niswander, 2012). The tissues produced using the self-organising technique have native tissue morphology and can be grown up to several centimetres (Baltich et al., 2010; Calve et al., 2004; Smietana et al., 2009).

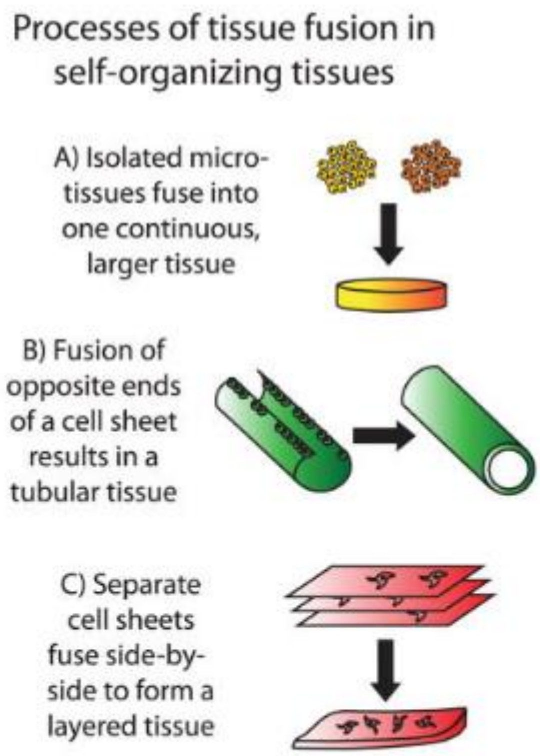

Figure 2-5 Mechanism involved in self-organising tissue engineering technique (Athanasiou et al.,, 2013)

The self-organising technique was first reported by Benjaminson et al. (2002), who produced the first-ever in-vitro meat using goldfish (*Carassius auratus*) skeletal muscle explant, which regenerated and rearranged itself without any matrix, and showed a growth rate of 79% from the initial state. The explant was identical to the goldfish skeletal muscle and resembled fish fillets in terms of appearance and odour (Benjaminson et al., 2002).

The self-organising technique is beneficial as it mimics skeletal muscles by retaining all the tissues which form the meat. Another benefit is its ability to produce a highly structured meat, unlike other methods. However, there are significant drawbacks with this method such as its susceptibility to undergo necrosis in the absence of blood supply (Dennis and Kosnik, 2000). Furthermore, IVM produced by this method requires the need for in-vivo blood supply/vascularisation and an excretory mechanism to expel metabolic waste (Bhat and Fayaz, 2011). A major drawback of the self- organising technique is its inability to produce highly structured meat since it produces non-structured and soft consistency meat, which is suitable for sausages, minced meat and burger patties (Bhat and Bhat, 2011)

2.5.2.2 Scaffold technique

'*A scaffold or matrix for a tissue engineering product refers to the ability to perform as a substrate that will support the appropriate cellular activity, including the facilitation of molecular and mechanical signalling systems, in order to optimize tissue regeneration, without eliciting any undesirable local or systemic response in the eventual host*' (Williams, 2008).

The scaffold technique is a tissue engineering technique, which uses three-dimensional structures made of hydrogels, as a substrate to grow cells or tissues of interest. This method has gained immense popularity in the last few years and is widely used in IVM research (Acevedo et al., 2018; Catts and Zurr, 2002a) because scaffolds can act as a matrix for cell adherence to produce edible skeletal muscle tissues or IVM. The scaffolds are made from hydrogels of natural or synthetic polymers, which are designed according to requirements, that are later seeded with cells of interest. These cell-laden scaffolds are immersed in nutrient-rich medium contained in a bioreactor. Under favourable conditions, these cells grow into myotubes, which eventually form myofibrils. On maturation of cells, the resultant muscle fibres are harvested as edible skeletal muscle tissue or IVM.

Scaffolds developed using natural and edible hydrogels such as collagen and gelatin can produce complex meat with 3D structures. Thus, collagen and gelatin-based scaffolds are widely used to grow skeletal muscle tissues.

2.5.2.3 Bioreactors

The production of IVM can be carried out either using scaffolds or by scaffold-free techniques, such as the hanging drop method or agitation bioreactor method as seen in Figure 2-6.

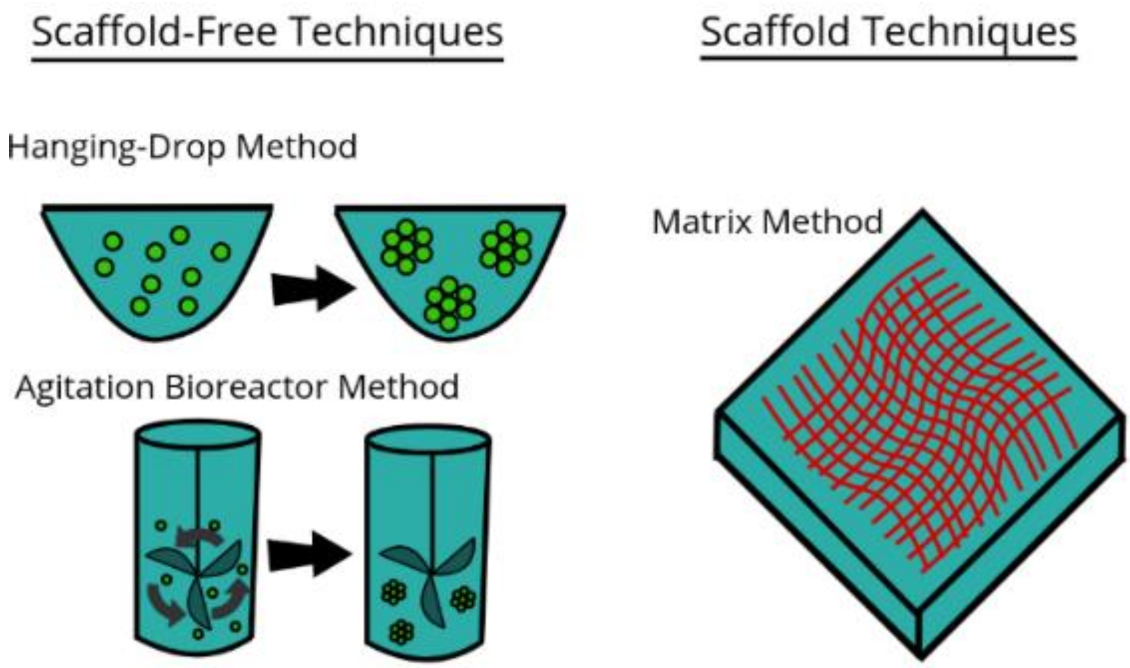

Figure 2-6 Scaffold free technique and scaffold techniques in tissue engineering, which can be used in IVM production (source: Declan Riordan [CC BY-SA 4.0 (https://creativecommons.org/licenses/by-sa/4.0)], https://commons.wikimedia.org/wiki/File:3d_cell_culture_(1).svg.)

Bioreactors, as seen in Figure 2-7, are large enclosed stainless steel units used for culturing cells in a sterile manner, as it provides a favourable environment for the proliferation of cells (Bhat and Bhat, 2011; Martin et al., 2004). The production of IVM is facilitated by biophysical factors such as agitation and shear that are achieved by the inclusion of bioreactors. Bioreactors are generally equipped with a media source, scaffolding system, oxygenation system and a plumbing system for the continuous inflow of media and outflow of metabolic wastes and recycled media. Here, the cells either suspended freely or are seeded onto a scaffold suspended in a bioreactor. The cells then undergo proliferation and differentiation to yield 3D muscle fibres, which can be potentially used as IVM.

The bioreactor can help with IVM production in several ways. Firstly, bioreactors help with the continuous suspension of culture media, so the cell culture is not deprived of the nutrient source. Secondly, it helps with agitating using a low shear, so that the suspended tissues are unaffected. Thirdly, it assists with adequate oxygen perfusion as oxygen gradient influences the cell viability and density. Fourthly, it assists with continuous contraction of cells, which eventually undergoes differentiation to produce myofibers.

There are several types of bioreactors that can be used for IVM production. This includes the rotating wall /vessel (Chen and Hu, 2006; Reichardt et al., 2013), stir tank/stirred (Tuomisto and Teixeira de Mattos, 2011), direct perfusion (Zhao et al., 2007), rotatory (Edelman et al., 2005), hollow fibre (Hambor, 2012; Whitford et al., 2014), wave mixed (Hami et al., 2004), rotatory bed (Reichardt et al., 2013), parallel plate (Peng and Palsson, 1996), fixed bed (Lennaertz et al., 2013) and Synthecon (Catts and Zurr, 2002a) reactors. However, a stir tank bioreactor is most commonly used in IVM production (Chu and Robinson, 2001; Fok and Zandstra, 2005; Rafiq et al., 2013; Serra et al., 2010; Xie et al., 2003).

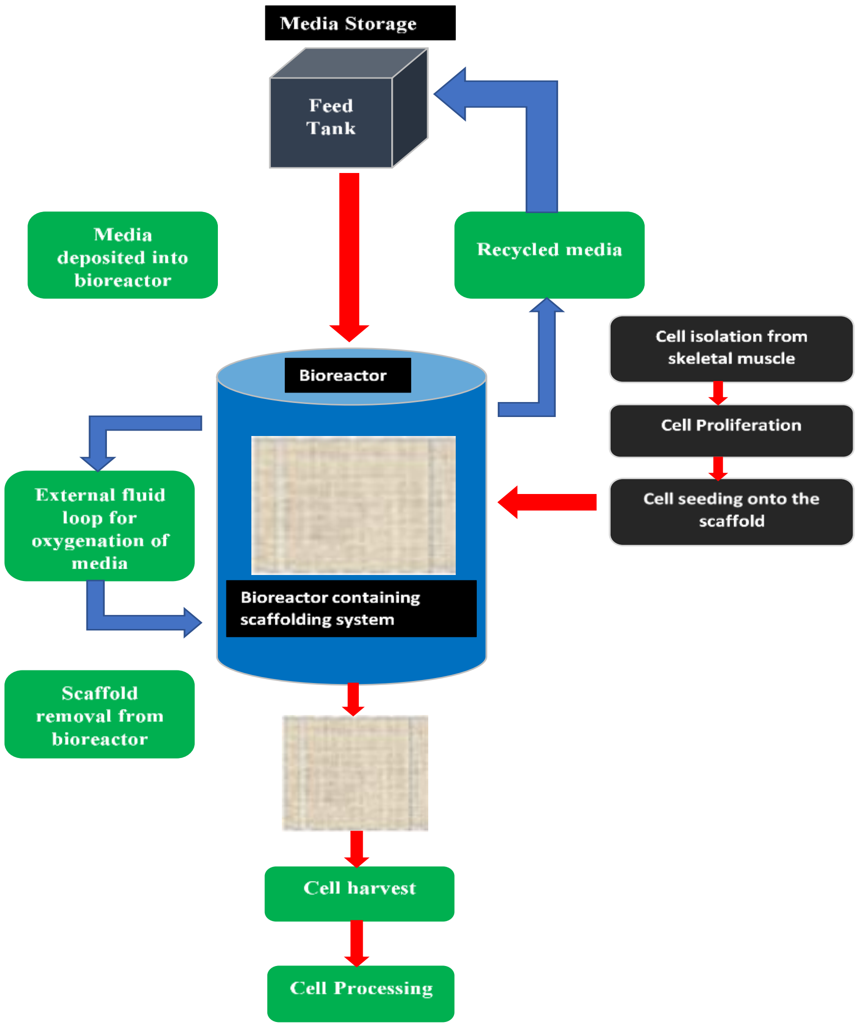

Figure 2-7 Schematic representation of IVM production (Gaydhane et al., 2018).

2.5.2.4 Stimulation of cells

In an in-vivo system, all the cellular process occurs naturally. These processes are carried out by nerve stimulation and electrical transmission/stimulation, with the help of extracellular matrix (ECM). However, this is not the case when cells are grown in-vitro. The challenge lies in mimicking the in-vitro environment like the in-vivo environment. Thus, in-vitro systems require external stimulation of cells, which is brought about in two ways, either by electrical or mechanical stimulation as described in sections 2.5.2.4.1 and 2.5.2.4.2.

2.5.2.4.1 Electrical stimulation

The electrical stimulations mimic the nerve stimulations, which assist with the formation of highly differentiated and functional skeletal muscle tissue. Typically, electrical stimulation in in-vitro studies is carried out by passing an electrical stimuli via salt bridges, which are dispensed in culture media (Song et al., 2007). The general set up of electrical stimulation units are shown in Figure 2-8. However, there are a few setbacks with this method, such as a limited working area, making it difficult to work with various cell types at a time. Besides, the media-bridge system is susceptible to temperature fluctuations and exchange of salts and ions during electrical stimulation of cells can result in alteration temperature, pH and salinity. Furthermore, the electrical stimulation system is incapable of running multiple chambers making it difficult to maintain sterility (Mobini et al., 2016).

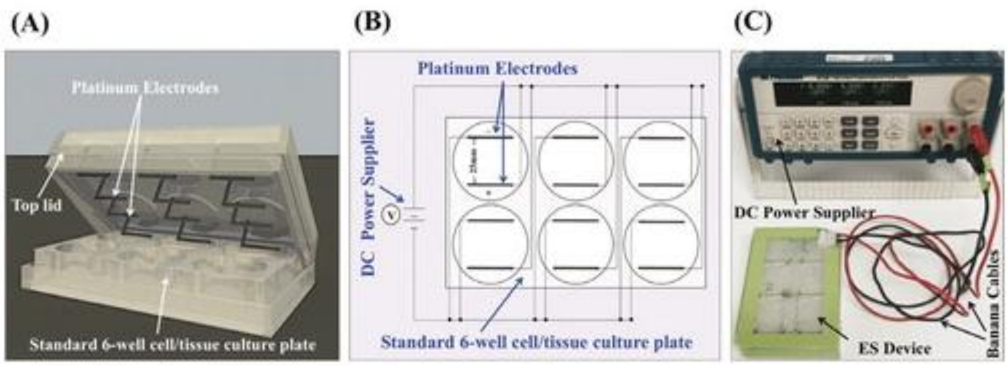

Figure 2-8 Set up of electrical stimulation, which is applied to cells to facilitate cell proliferation (Mobini et al., 2016)

Electrical stimulation of cells during its growth is critical for IVM production for several reasons. Firstly, electrical stimulation induces neuronal activity in the mature muscle fibres (Wilson and Harris, 1993), and can be carried out by applying an electrical stimuli (Bach et al., 2003). Secondly, electrical stimulation helps in accelerating the maturation of myotubules to develop early cross striations in C2C12 murine myoblasts. Thirdly, electrical stimulation helps with neuronal activity by initiating contraction and differentiation of myotubules to eventually form myofibres (Bhat and Bhat, 2011).

2.5.2.4.2 Mechanical stimulation

Mechanical stimulation is a biophysical stimulus that can be provided during myogenesis (Vandenburgh and Karlisch, 1989), as it influences gene expression, protein synthesis and total RNA/DNA content. Furthermore, it also helps with myofiber composition, cell number and muscle fibre diameter (Cai et al., 2017).

Mechanical stimulation of cells also helps with IVM production in several ways. Firstly, it helps by applying mechanotransduction (a process through which cell sense and respond to mechanical stimuli by converting them to biochemical signals that elicit specific cellular responses), which alters the cell proliferation and differentiation rates (Burkholder, 2007). Secondly, it helps with the fusion, alignment and maturation of the myotube. Thirdly, it helps with the proliferation and differentiation of muscle cells (Langelaan et al., 2010), muscle alignment (Vandenburgh et al., 1999) and muscle growth and maturation (Rangarajan et al., 2014; Tidball, 2005).

There are various methods of mechanical stimulation of cells (Brown, 2000). Here, the mechanical force is generated by using a perfusion bioreactor. When these mechanical forces are applied, this leads to perturbations in muscular protein conformation. This results in the exposure of hidden binding sites, which indirectly increases the signalling process in cells entrapped in the scaffold (Polacheck and Chen, 2016; Ridley, 2003). However, mechanical overloading can result in deformation, remodelling of cell, and even affect cellular functions (Urrutia et al., 2017).

2.5.2.5 3D bioprinting

3D bioprinting is a novel method to create three-dimensional scaffolds of different hydrogel compositions. There are different types of printers available based on the technology, such as laser-assisted printing (Godoi et al., 2016), as well as extrusion-based and inkjet-based 3D printing (Jammalamadaka and Tappa, 2018; Liu and Yan, 2018). These 3D printers can efficiently create complex shapes of scaffolds with high resolution (Liu and Yan, 2018). However, extrusion-based 3D printing is the most commonly used 3D printer (Jammalamadaka and Tappa, 2018).

There two main methods of 3D printing involve the use of either cellular scaffolds with cell-laden bioink or acellular scaffolds with hydrogels (Jammalamadaka and Tappa, 2018). There are several studies on 3D printings for tissue engineering and regenerative medicine purposes (Cui et al., 2018; Cui et al., 2016; Deng et al., 2017; Merceron et al., 2015; Murphy and Atala, 2014), but not many on the 3D printing skeletal muscle cells for meat purposes (Acevedo et al., 2018; Catts and Zurr, 2002a). In my current study, we have used the extrusion-based 3D printer (Allevi 2) to create acellular scaffolds. The detailed information of scaffold printing is covered in the methodology section 3.2 onwards

2.5.2.5.1 3D printing of scaffolds

Figure 2-9 Allevi 2 3D bioprinter used in our study to print GelMA, collagen hyaluronic acid scaffolds (Allevi, 2019).

In our study, extruded scaffolds were developed using the Allevi 2 bioprinter, as seen in Figure 2-9. It is a Fused Deposition Modelling (FDM) bioprinter that runs on a compressed air pneumatic system (Allevi, 2019). It has two extruders, where the first extruder extrudes bio-inks, and the other extruder is used for photocuring (visible or UV light) extruded scaffolds. The hydrogel/ bioink is dispensed in syringes with needles of 0.3mm nozzle diameter to extrude scaffolds of synthetic polymers.

The bioprinter works on a three-dimensional computer-aided design (CAD) software such as Slicr and Repertoire host, which helps in designing scaffolds. The 3D CAD models of the desired scaffolds are sliced into 2D cross-sections, to adjust printing parameters such as speed, in-fill density, gauges, nozzle diameter, print temperature, number of layers, layer height and air pressure. Software's such as Slicr and repertoire host combines the two-dimensional cross sectionals of the scaffold to form a computer-aided three-dimensional structure. The bioprinter has a triaxial system (x, y, z), which allows scaffolds to be printed into desired shapes, which are then cured using built-in UV light to carry out crosslinking reactions.

2.5.2.5.2 Hydrogels

Hydrogels are three-dimensional polymer gels which is made up of water-soluble polymers, that are held together by water-insoluble cross-linkages. In other words, hydrogels are formed by the crosslinking (refer section 1.5.2.5.2.1 and 1.5.2.5.2.2) of homopolymers or copolymers to give 3D structures with unique mechanical and chemical characteristics. Generally, most hydrogels swell and increase their weight when added to water due to their imbibition property. These hydrogels can be further classified into chemical or physical hydrogels based on the crosslinking mechanisms (Chang et al., 2010; Lee et al., 2008; Naahidi et al., 2017). Physical hydrogels are not permanent, whereas chemical hydrogels are permanent.

Hydrogels are either natural or synthetic in nature, and the natural hydrogels are comprised of polysaccharides and proteins and are usually found in the tissues such as agarose, gelatin, elastin, alginate, cellulose chitosan, fibrin, collagen and Matrigel (Bian and Bursac, 2009; Gawlitta et al., 2008) and hyaluronic acid. Natural hydrogels are more preferred due to their extracellular matrix-like structure, which enables cell growth, solute transport, cell binding and other cellular behaviours (Ahadian et al., 2015). Further information on collagen, gelatin and hyaluronic acid are listed in section 1.5.2.5.3.

Whilst, synthetic hydrogels are usually made of polyethene glycol (PEG) (Gilbert et al., 2010), poly-(L-lactic acid) (PLLA) and polylactic-glycolic acid (PLGA) (Levenberg et al., 2005), polydimethylsiloxane (PDMS) (Bian and Bursac, 2009), and poly(vinyl alcohol) (PVA) (Jiang et al., 2011). Semi-synthetic hydrogels such as gelatin methacrylate/gelatin methacryloyl (GelMA) have been used previously to culture cells

(Nichol et al., 2010). Besides, these synthetic hydrogels can be used in the production of highly structured 3D IVM as it can facilitate cell entrapment and cell growth.

Hydrogels, in general, are extensively used in biomedical sciences applications (Ahadian et al., 2015; Deligkaris et al., 2010; Hoffman, 2012; Miri et al., 2018; Naahidi et al., 2017), as it mimics extracellular matrix (Miri et al., 2018) and due to its biocompatibility, biodegradability, density and crosslinking properties. Besides, these hydrogels offer a promising approach for skeletal muscle tissue engineering for many reasons. Firstly, it allows dense cell entrapment uniformly in the hydrogel scaffold (Bian and Bursac, 2008). Secondly, it assists with myotube alignment due to in-vivo like environment.

The major drawback with hydrogels is its instability, but it can be managed by co-culturing cells that produce extracellular matrix, which stabilises the matrix while the hydrogel degenerates (Langelaan et al., 2010). The reproducibility and uniformity of the gels can be adjusted by electrospinning, but this may result in non-uniform distribution of cells.

2.5.2.5.2.1 Crosslinking reaction

In chemistry, cross-links are referred to bonds that connect one polymer chain to another through covalent bonds or ionic bonds. These cross-linkages are either formed by covalent (Edman et al., 1980; Langer and Folkman, 1976) or non-covalent interactions (De Jong et al., 2001; Xiao and Yang, 2006) to form either chemical gels or physical gels respectively.

The process of crosslinking can be carried out in two ways, either physically or chemically. Chemically crosslinking is carried out by either polymerization, chain-growth polymerization, sulphur vulcanization or by chemical reactions such as addition and condensation irradiation. It can also be performed by irradiation using high energy x-ray, e-beam and gamma rays. On the other hand, physical crosslinking is conducted by ionic interactions, crystallization, stereo complex formation, and protein interaction. Crosslinking is vital as it affects physicochemical properties of polymers such as elasticity, viscosity, swelling, solubility and strength of gels (Maitra and Shukla, 2014). A detailed description of crosslinking of polymers is beyond the scope of this study but can be read in the article referenced (Hennink and van Nostrum, 2012).

2.5.2.5.2.2 Polymerisation reaction

Polymerisation is a crosslinking method where several monomers (homopolymers or copolymers) units react together chemically to form three dimensional polymers. Polymerisation is essential for hydrogels as it determines the physicochemical properties based on its monomers. A detailed description of the polymerisation reaction is beyond the scope of this study, but further information is available in the study referenced by (Maitra and Shukla, 2014).

2.5.2.5.3 Types of hydrogels

2.5.2.5.3.1 Collagen hydrogel

Collagen is a naturally occurring protein that makes up 25% of the protein content in mammals (Harkness, 1961). Besides, it is the main component of extracellular matrix (Ahadian et al., 2015), which provides *in-vivo* like environment that enables cell encapsulation, cell binding and integrin signalling (Levental et al., 2009). In addition, collagen also provides exceptional crosslinking ability (Lee et al., 2001), low antigenicity, biodegradability (Maeda et al., 1999) and higher biocompatibility (Lee et al., 2001). Therefore, collagen hydrogels are widely used as a scaffold in tissue engineering (Ahadian et al., 2015).

Collagen hydrogels have a few drawbacks. Firstly it is soft and susceptible to degradation, but can be managed either by increasing the amount of collagen or by chemically modifying it to prevent degradation (Roberts and Martens, 2016). Secondly, it has the ability to trigger an immune response occasionally, which can affect cell culture. Thirdly, the usage of collagen hydrogels, in the long run, is not an economical option (Dubey and Deng, 2018). Finally, collagen demerits include thermal instability, low mechanical strength and susceptibility to contaminations (Ahadian et al., 2015; Lee et al., 2001).

2.5.2.5.3.2 Gelatin hydrogels

Gelatin is a natural polymer, which is obtained by collagen hydrolysis. It is an economical, temperature-responsive polymer with high cell adhesiveness (Ibusuki et al., 2003). In addition, gelatin hydrogels are often functionalized with a cross-linkable component like methacryloyl group, which is crosslinked by photoinitiators (Van Den Bulcke et al., 2000) to enhance hydrogels stability (Ahadian et al., 2015). However, most gelatin hydrogels are prone to hydrolysis, but it can be managed by chemical modification (Lee and Mooney, 2001).

Gelatin methacryloyl (gelMA) is a modified form of gelatin, which is obtained by chemical crosslinking. It is one of the most widely used hydrogels as it offers excellent biocompatibility, physicochemical properties, printability and is cost-effective (Lee et al., 2016; Nichol et al., 2010). Consequently, GelMA has been widely used in cell/tissue culture studies as an extracellular matrix due to its exceptional cell binding characteristics (Van Vlierberghe et al., 2011), migration, cell differentiation and proliferation (Roberts and Martens, 2016). Nevertheless, there are a few demerits such as low mechanical strength (Colosi et al., 2016; Liu et al., 2017; Yin et al., 2018), as well as reduced cell distribution and migration (Nichol et al., 2010). However, this can be managed by combining gelMA with hyaluronic acid or and silk fibroin (Xiao et al., 2019).

2.5.2.5.3.3 Hyaluronic acid hydrogels

Hyaluronic acid (HA) or hyaluronan is a polysaccharide abundantly found in connective, epithelial and neural tissues. It is responsible for the formation of extracellular matrices with the help of glysosaminoglycans. Furthermore, it also helps with cell proliferation, migration, cell behaviours and other cellular functions. However, HA requires purification before hydrogel preparation in order to eliminate impurities and toxins.

Hyaluronic acid has been used in many genetic engineering applications (Choi et al., 1999; Duranti et al., 1998; Radomsky et al,, 1996) as it is biocompatible with cells and mimics in-vivo conditions. In addition, it helps with angiogenesis in engineered tissues (Pardue et al., 2008) to promote vascularisation. Thus HA has been widely used in the biomedical and tissue engineering research for over 30 years (Burdick and Prestwich, 2011).

3. Chapter 3. MATERIALS AND METHODS

3.1 Consumer perception study on IVM

3.1.1 Ethics Statement

This study was approved by the Auckland University of Technology Ethics Committee (AUTEC 19/68).

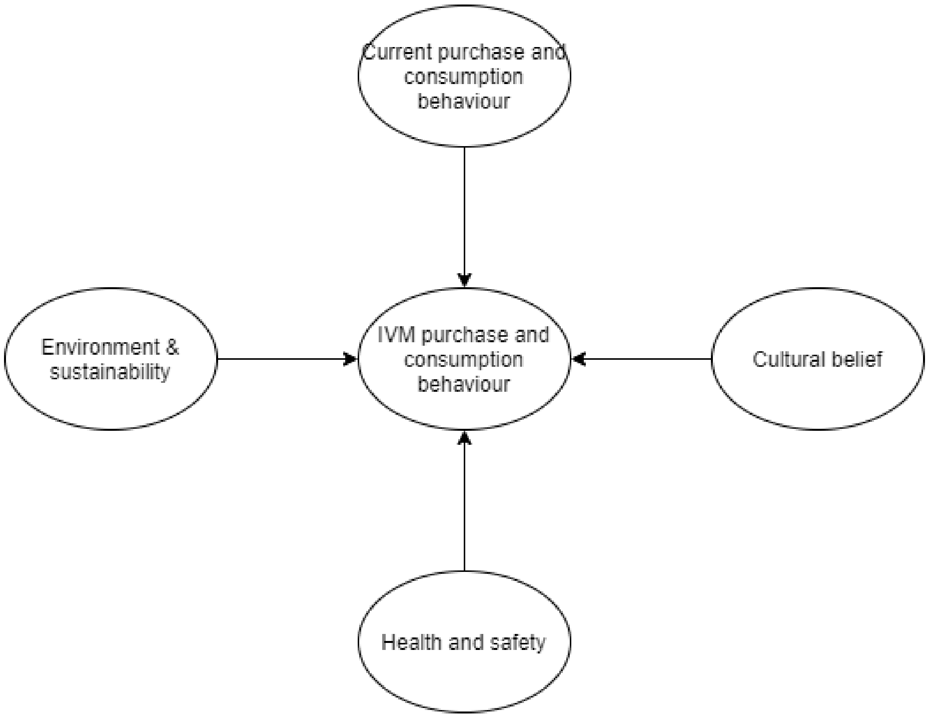

Figure 3-1 Conceptual model used in our study to test the hypothesis.

3.1.2 Research design

To explore the perception of IVM by New Zealand consumers, quantitative research was conducted using an online survey as a research tool that is widely used in consumer science studies. Furthermore, online surveys are considered a platform for the collection of large amounts of anonymous responses.

In addition, this study also attempts to measure behavioural intentions such as WTT and WTB, since the commercial success of IVM, as a sustainable alternative, lies in the WTT, WTB, and WTE IVM. These approaches were used with the US, the European and Asian consumers to determine their WTT, WTB and WTE IVM (Bryant and Barnett, 2019b; Bryant et al., 2019c; Mancini and Antonioli, 2019; Wilks and Phillips, 2017).

3.1.3 Procedure

3.1.3.1 Online survey recruitment

Consumer views were collected through an online survey in New Zealand, and the survey was administered using the Qualtrics Software (Utah, United States). The survey was open to all New Zealanders, irrespective of their eating habits. The survey was administered in English, between March to September 2019. It was open to every New Zealander, aged between 18 to 65 years old. Most of the participants were recruited in person, whilst the rest were recruited online through an advertisement posted on social networking sites, such as Facebook, Instagram and LinkedIn.

3.1.3.1.1 Participants

Participation in the research was entirely anonymous, voluntary and confidential. Participants were under no obligation to take part in the study and had the freedom to withdraw at any stage without any further questions. The participants were given a coffee voucher as a token of appreciation. The eligibility criterion to take part in the study were respondents over the age of 18 and must be living in New Zealand.

A total of 265 participants were recruited, but once incomplete surveys were omitted, 206 completed surveys were obtained for further analyses.

3.1.3.2 Online survey procedure

The questionnaire was mostly accessed either by scanning the QR code/anonymous link generated by Qualtrics or through links shared on Facebook and LinkedIn. As previously mentioned in section 3.1.5.1.1 no participant was under obligation to take part in the study, thus all participants used a checkbox to indicate their consent to take part in the study, before the answering the survey questionnaire.

Before the commencement of the survey, all participants were briefed about the study and were given some information (refer appendix 2) about in-vitro meat to educate, rather than influence consumers. Provision of information has been proven to improve the consumer perception (Bekker et al., 2017; Verbeke et al., 2015; Verbeke et al., 2015b) and also result in increasing WTT (Flycatcher, 2013; Verbeke et al., 2015b).

Participants were first asked demographical questions, followed by questions on eating behaviour and meat consumption. In addition, questions on participants' familiarity, as well as their willingness to try/buy IVM if commercially available, were also included. Participants were asked to rate their answers on a 5-point Likert scale (refer appendix 2). Furthermore, the questionnaire also included questions on other aspects of IVM such as marketing, regulatory, ethical and religious views that indirectly affect the perception.

The data from the survey were automatically saved on the Qualtrics website and were extracted on completion of data collection. The collected raw data were analysed using XLSTAT (version 2018.5) (Addinsoft, USA) and R Studio (version 1.1463) operating with R-3.6.1packages.

3.1.4 *Data analysis*

The conceptual framework model as shown in Figure 3-1 used in this study was developed to understand the effect of New Zealand consumer perception of IVM purchase and consumption behaviour. As stated in the hypothesis (section 3.13), consumers' views on environment and sustainability, health and safety, cultural beliefs and consumers' current purchase and consumption behaviour of meat play an important role in purchase intention of IVM. Consequently, most studies in the IVM literature examine the consumer perception of IVM and its effects on purchase likelihood (Bekker et al., 2017; Bryant and Barnett, 2019b; Bryant and Dillard, 2019d; Bryant et al., 2019c; Gómez-Luciano et al., 2019; Hocquette et al., 2015; Laestadius and Caldwell, 2015; Mancini and Antonioli, 2019; Slade, 2018; Verbeke et al., 2015; Verbeke et al., 2015b; Wilks and Phillips, 2017). However, the framing of the research objectives, hypothesis and variables examined vary with each study.

Previously studies on IVM used other regression models such as logistic regression, mixed logic and multiple regression models (Bryant et al., 2019c; Gómez-Luciano et al., 2019; Slade, 2018; Verbeke et al., 2015b; Wilks and Phillips, 2017) and other statistical methods like ANOVA (Bryant and Dillard, 2019d; Gómez-Luciano et al., 2019). In this study, PLS-PM was used since it was a more suitable method for studying complex multivariate relationships between latent and observed variables (Sanchez, 2013).

The conceptual model was validated and measured using Goodness of Fit statistics, and measures the overall model fit for PLS-PM. The internal validity of the variables was assessed using Cronbach's α and using Kaiser Meyer Olkin (KMO) values. The Cronbach's alpha value measures the internal consistency and scale reliability. Whereas, KMO values measure the sampling adequacy for each variable and for the model. The above statistical tests are essential for justifying results and confirming the conceptual model. However, at this point, no inter-item correlation was performed due to the numerous variables involved in this study.

Since PLS-PM does not hold any distribution assumption (Vinzi et al., 2010), the resampling task was performed to validate and obtain further data on the variability of parameter estimates (Stephanie, 2018). The accuracy of the PLS-PM model was obtained by bootstrapping. This is a non-parametric procedure that measures the significance of the PLS results such as coefficients, Cronbach's alpha, HTMT and R^2 value (Davison and Hinkley, 1997; Efron and Tibshirani, 1986). The R^2 value of the conceptual model in this study was found to be 0.777. Furthermore, PLS-PM analysis was carried out using the PLSPM package version 0.4.9 (Sanchez, 2013) by R Studio (RStudio, USA) (version 1.1463) operating with R-3.5.1.

3.2 *Preliminary trials of in-vitro meat (IVM) production*

In this study, cell culture for preliminary trials of IVM production was carried out using murine C2C12 cells. These are immortalized mouse myoblast cell line which is capable of rapid proliferation and differentiation under different conditions. Generally, C2C12 myoblast cells are used in myogenesis studies in biomedical research (Acevedo et al., 2018; Krieger et al., 2018; Kroehne et al., 2008; Schuster et al., 2017).

Murine C2C12 myoblast cells were grown on 3D printed scaffold of various composition (gelMA, collagen and hyaluronic acid) and different number of layers (2, 4 and 8 layers) (Table 3-2). The presumption here was, if C2C12 cells can be successfully cultivated on scaffolds, then it will be possible to grow skeletal muscle cells on scaffolds too, which can be potentially used to produce IVM. However, cultivation of skeletal muscle cells is possible only if favourable culture conditions are provided since the growth requirement for C2C12 cells are quite different from that of skeletal muscle cells.

3.2.1.1 *Hydrogel preparation*

3.2.1.1.1 *Preparation of 8% gelMA solution*

Lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP), a crosslinking agent (0.02g) was weighed (Metler toledo, New Zealand) and transferred into a vial. Then 2ml of 0.5N Phosphate Buffered Saline (PBS) (Sigma Aldrich, New Zealand) (pH=7.4) was added at 25℃ and mixed thoroughly to avoid any residues. To this vial, GelMA (Allevi, New Zealand) (0.032g) was added and covered by an aluminium foil to prevent LAP from crosslinking under normal light. The mixture was stirred continuously using a magnetic bead stirrer on a hot water bath (Heidolph, New Zealand) at 60℃ for 30 mins, or until gelMA was completely dissolved.

3.2.1.1.2 Preparation of 1% collagen solution

About 0.04 g of collagen (New Zealand Shoe and leather research association - LASRA, New Zealand) was measured and transferred into a Falcon tube. Later, it was dissolved in 1 ml of 0.5N acetic acid (pH=2.8.3) and swirled using a vortex (Ohaus, USA). The mixture was then put into an ice bath and left overnight to ensure that the solution was completely dissolved. Later, 1% collagen sample was mixed with 8% gelMA and LAP solution to be used as a bio-ink.

3.2.1.1.3 Preparation of 1 % hyaluronic acid solution

Hyaluronic acid (Sigma Aldrich, New Zealand) was weighed (0.02 g) and dissolved in 1ml of cold phosphate-buffered saline (PBS) (pH=7.4). The solution was mixed with 8% gelMA and Lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) solution to be used as a bio-ink.

3.2.1.2 3D printing of scaffolds

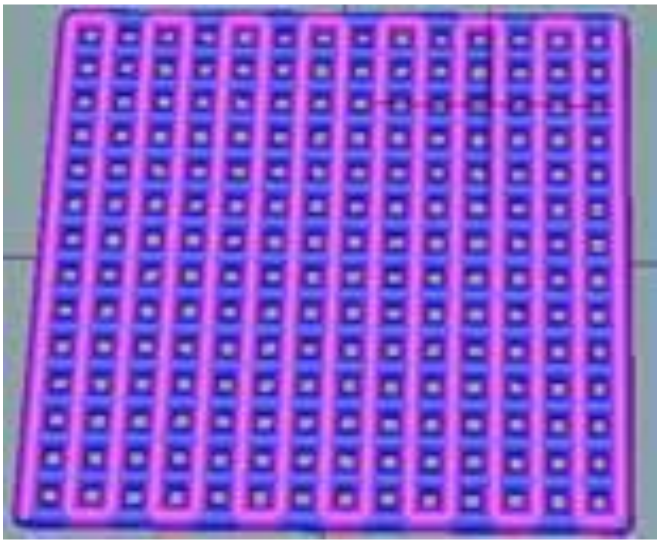

Figure 3-2 A 3D model of a 2-layer model used in the stud, first layer of scaffold is coloured blue and the second is coloured pink.

All hydrogels (gelMA, gleMA+collagen and gelMA+hyaluronic acid) were dispensed into a disposable syringe based on compositions (Table 3-1), and later the hydrogels were loaded into extruder 1 in the Allevi2 bioprinter (Allevi, New Zealand) to print scaffolds.

A typical 3D model of a 2-layer scaffold matrix is shown in Figure 3-2, where the first layer of the scaffold is juxtaposed with another flipped layer to form a mesh with pores in between. All the 3D printed scaffolds used the following printing parameters:12 PSI pressure, 27G needle, 3mm/s speed, 0.21mm layer height, and cured/photo-crosslinked for 3 mins time on each side.

In this study, 32 scaffolds of 3 different hydrogel compositions were extruded on to a clean glass slide (Table 3-1), and these were classified as mesh scaffolds. Apart from the scaffolds mentioned above, some hydrogels were directly pipetted onto clean glass slides to form convex-shaped scaffolds, these were classified as pipette scaffolds. Both, gelMA mesh and pipetted scaffolds were cured (crosslinked) using UV light (SUNUV, New Zealand) at 24kW for 3 mins on each side. The scaffolds were left to dehydrate overnight using vacuum drier (Labline, India). Once the scaffolds were completely dry, it was detached from the slide and stored in an air-tight container until the cell seeding stage.

Table 3-1 Composition of various hydrogel scaffolds used in the study.

Scaffold composition	Pipette scaffold	2 -layer	4 -layer	8 -layer
8% gelMA (mesh)	3	3	3	3
8% gelMA + 1% collagen (mesh)	0	3	2	3
8% gelMA +1% hyaluronic acid (HA) (mesh)	3	3	3	3

3.2.1.3 Cell culture methodology

3.2.1.3.1 Cell culture medium

The murine C2C12 myoblast cells were cultured using the Dulbecco's modified eagle medium (DMEM) as proliferation media, which was supplemented with 10% foetal bovine serum (FBS) (ThermoFisher Scientific, New Zealand). About 1% penicillin/streptomycin (ThermoFisher Scientific, New Zealand) and 1% fungizone (ThermoFisher Scientific, New Zealand) were added to the media as antibacterial and antifungal agents. Whilst, DMEM supplemented with 2% horse serum (ThermoFisher Scientific, New Zealand) and 1% penicillin/streptomycin was used as differentiation media.

3.2.1.3.2 Cells and Scaffold treatments

Murine C2C12 myoblast cells were cultured for two weeks prior to seeding on scaffolds at the University of Canterbury (UoC). The cells were later trypsinised (ThermoFischer, New Zealand), pelleted and resuspended in proliferation media then counted with Trypan Blue (Gibco, T8154-20ML). Whereas, all the scaffolds were placed in 12 well-plates and rehydrated with PBS, which were then sterilized by UV irradiation for one hour while being immersed in PBS.

GelMA and cellulose scaffolds

Thirty-two gelMA scaffolds of different compositions (Table 3-1) were added to 12 wells cell culture plates. A suspension of $6x10^4$ C2C12 cells (21 passage[19]) was added to each well. Apart from gelMA, 6 cellulose scaffolds were added into a 96-well plate. To these, a suspension of 7.6uL of C2C12 cells (23 passage) was added to each well. Besides, another set of $6x10^4$ C2C12 cells (21 passage) was added to 12 wells of the control plate to follow changes in cell proliferation and differentiation stages of the cell-laden scaffold and control visually. All the cell-laden scaffolds and control were incubated at 37℃ in a 7.5% CO_2 atmosphere. No external stimulation was applied to cells on the scaffolds to stimulate growth.

Some of the gelMA scaffolds were damaged at the time of cell seeding at University of Canterbury (UoC), consequently, six cellulose scaffolds were offered by UoC as a replacement due to shortage of scaffolds. These cellulose scaffolds were used to perform cell viability tests using Alamar blue assay and trypan blue staining. Literature shows that cellulose scaffolds can be used for cell viability tests due to its biocompatibility (Fang et al., 2009), but there is no evidence for the use of C2C12 cells on cellulose scaffolds.

3.2.1.3.3 Micrography of cell-laden scaffolds

All the cells were imaged using differential interference contrast microscopy (DIC) microscopy using a Nikon ECLIPSE TS2 on a predominantly x10 magnification, and x20 images were taken. Cell images were taken at regular intervals (0hr, 3hr 13h,

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[19] Cell passage number – is a number of times the culture has been sub cultured.

21 Passage- here 21 refers to the 21st time the cell culture has been harvested and reseeded into daughter cell culture(Public Health England, 2017).

35h, 57h, 80h, 105h, 128h, 151h, and 175 hours) to observe cellular processes like attachment, proliferation and differentiation inside the scaffolds.

3.2.1.3.4 Cells viability assay

Alamar blue

Alamar blue is a reagent, which quantitatively measures proliferation in cells. It is a direct indicator of cell health. It is also used in cell viability assays, in-vitro cell-toxicity assays and monitoring cell growth. Alamar blue is a water-soluble, cell-permeable, non-toxic, highly sensitive reagent, which consists of resazurin, a weak fluorescent blue indicator dye. Resazurin, a redox indicator undergoes a colorimetric change in response to cellular metabolite reduction to form, resorufin, a pink coloured dye which is highly fluorescent. The intensity of fluorescence is directly proportional to the number of viable and respiring cells. The Alamar blue assay protocol is summarized in Figure 3-3.

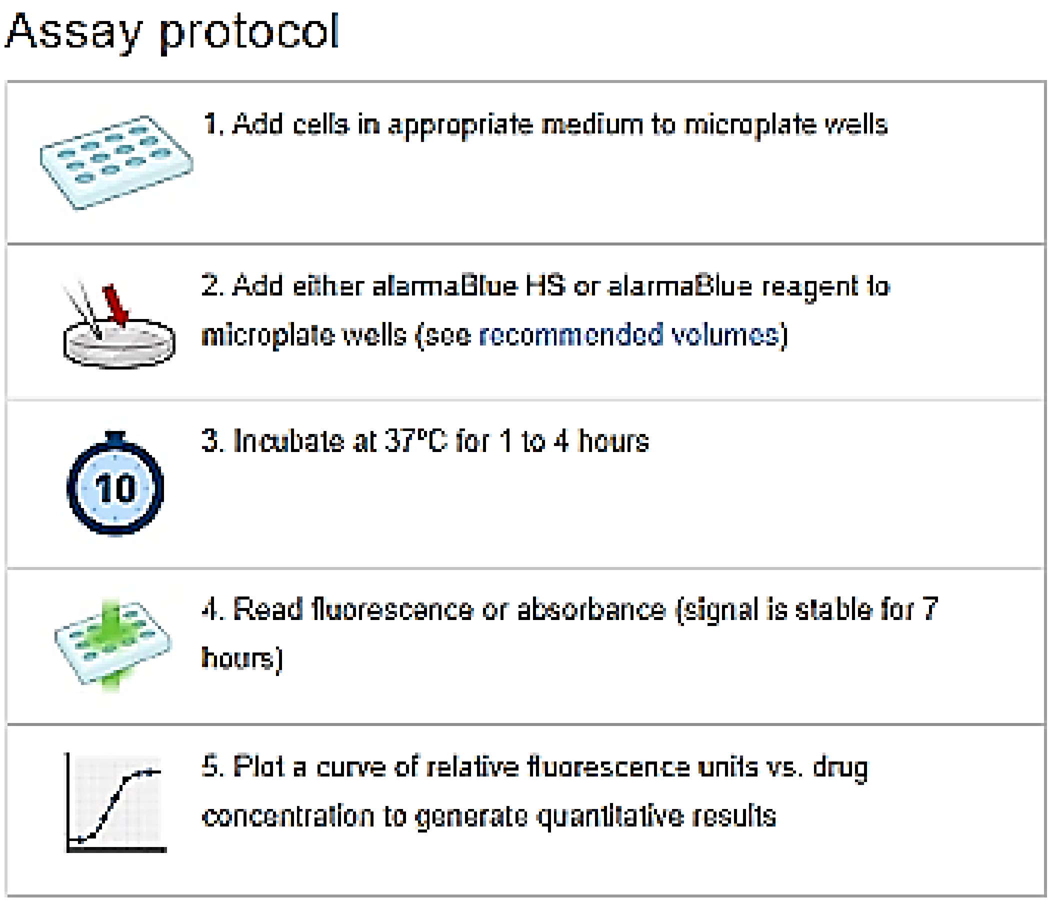

Figure 3-3 General protocol for Alamar blue assay (source: Thermofischer Scientific)

Trypan blue

Trypan blue is a negatively charged dye used to identify dead cells in cell culture studies. The dye penetrates through compromised cell membranes of the dead cells, enters the cytoplasm and imparts a blue colour to the dead cells when observed under light microscopy. Live cells do not allow trypan blue to pass through the porous cell membrane. Thus, this method helps in distinguishing the dead cells and quantifying live cells. Furthermore, trypan blue also helps reduce the likelihood of false positives in flow cytometry. The general procedure of trypan blue staining is summarized in Figure 3-4.

Protocol

The following procedure will enable you to accurately determine the cell viability. Cell viability is calculated as the number of viable cells divided by the total number of cells within the grids on the hemacytometer. If cells take up trypan blue, they are considered non-viable.

1. Determine the cell density of your cell line suspension using a hemacytometer.
2. Prepare a 0.4% solution of trypan blue in buffered isotonic salt solution, pH 7.2 to 7.3 (i.e., phosphate-buffered saline).
3. Add 0.1 mL of trypan blue stock solution to 0.1 mL of cells.
4. Load a hemacytometer and examine immediately under a microscope at low magnification.
5. Count the number of blue staining cells and the number of total cells. Cell viability should be at least 95% for healthy log-phase cultures.

% viable cells = [1.00 – (Number of blue cells ÷ Number of total cells)] × 100

To calculate the number of viable cells per mL of culture, use the formula below. Remember to correct for the dilution factor.

Number of viable cells × 10^4 × 1.1 = cells/mL culture

Figure 3-4 General protocol for Trypan blue staining assay (source: ThermoFischer Scientific.

4. Chapter 4. RESULTS AND DISCUSSION

4.1 *Perception of IVM*

4.1.1 Sociodemographic characteristics

Sociodemographic characteristics of the sample population involved in this study are summarized in Table 4-1. The demographic factors included gender, age, qualification, income, nationality, religious, eating habits and frequency of meat consumption. Most of the respondents belonged to the 18-25 years age group (73.79%), with more women participants (61.17%), meat-eaters/non-vegetarians (69%), and participants who consumed meat most of the time (48.54%).

Table 4-1 Sociodemographic characteristics of the sample by number and percentage of respondents.

Variable	Category	Number	Percentage
Age	18-25	152	73.79%
	26-35	33	16.02%
	36-45	11	5.34%
	46-55	5	2.43%
	56-65	4	1.94%
	Prefer not to say	1	0.49%
Gender	Male	77	37.38%
	Female	126	61.17%
	Prefer not to say	3	1.46%
Qualification	High School	80	38.83%
	Diploma or certification	27	13.11%
	Bachelor’s degree	56	27.18%
	Master’s degree	22	10.68%
	Postgraduate degree	19	9.22%
	Prefer not to say	2	0.97%
Income	<$20,000 per year	62	30%
	Approx. $50,000 per year	19	9%

	$50,000 - $70,000 per year	16	8%
	$70,000 - $90,000 per year	22	11%
	$90,000 – $120,000 per year	14	7%
	>$120,000 per year	18	9%
	Prefer not to say	55	27%
Respondents nationality	New Zealander	59	29%
	Indian	21	10%
	Chinese	21	10%
	Other nationalities	78	38%
	Prefer not to say	27	13%
Religious	Yes	90	43.69%
	No	96	46.60%
	Prefer not to say	20	9.71%
Eating habits	Vegetarian	15	7%
	Non-vegetarian	141	69%
	Flexitarian	30	15%
	Pescatarian	2	1%
	Others	16	8%
	Prefer not to say	1	0%
Frequency of meat consumption	Always	44	21.36%
	Most of the time	100	48.54%
	Sometimes	34	16.50%
	Rarely	12	5.83%
	Never	16	7.77%
N = 206			

4.1.2 Data analysis of the conceptual model

4.1.2.1 Validity testing of means, KMO and Cronbach's alpha

4.1.2.1.1 Means

The mean and standard deviations of variables in the conceptual model are in Table 4-2. The mean values of most variables are in the range between 2-3 on a 5-point scale, which corresponds to poor perceptions and purchase intentions. Poor perceptions and purchase intentions have been attributed to consumer reluctance towards novel food products, especially if it is genetically modified due to techno scepticism (Verbeke et al., 2015), food neophobia (Bryant et al., 2019c; Gómez-Luciano et al., 2019), and disgust due to unnaturalness (Bryant et al., 2019a; Bryant and Dillard, 2019d).

Table 4-2 Variables extracted from the conceptual framework along with their mean, SD, KMO and Cronbach's alpha values

Factors	Mean	SD	Cronbach's Alpha	KMO
Environment & Sustainability	3.18	1.18	0.861	0.854
Health & Safety	2.72	0.95	0.573	0.714
Cultural Belief	2.05	1.08	0.622	0.749
Current Purchase Behaviour & Consumption	2.39	1.14	0.692	0.551
IVM Purchase	2.93	1.01	0.634	0.603

*The mean scores as seen in the table are based on 5-point Likert scale

*Kaiser Meyer Olkin measures the sampling adequacy.

4.1.2.1.2 Reliability

The factors of the conceptual model were measured for reliability using the Cronbach's alpha value, which tested the subscales for coherent dimensions (Streiner, 2003). Alpha values between 0.5-1 are considered as reliable. Values in this study ranged between 0.57-0.86 (Table 4-2), which indicates an exceptionally good consistency (Bryant et al., 2019a; Stephanie, 2014).

4.1.2.1.3 Factor analyses

The Kaiser Meyer Olkin (KMO) values have been used as a measure of sample adequacy for each factor in the conceptual model (Ismail and Shariff, 2008). Research showed that a KMO value greater than 0.5 was considered a good sample size (Kaiser, 1974). Results from this study (Table 4-2) showed KMO values between 0.50 to 0.90, meaning that it was acceptable (Stephanie, 2016).

Table 4-3 The β-coefficients and p-values of all variables in the conceptual framework showing the effect of all variables on purchase and consumption behaviour.

	Variables	β - coefficients	P-value
1.	Current purchase behaviour and consumption→IVM purchase	0.097	Pr > |t| = 0.034
2.	Cultural beliefs→IVM purchase	0.516	Pr > |t| > 0.001
3.	Health and Safety→IVM purchase	0.316	Pr > |t| < 0.001
4.	Environment and sustainability→IVM purchase	0.100	Pr > |t| = 0.036

4.1.3 Partial Least Squares Path modelling (PLS-PM): Structural model assessment

PLS-PM analysis was performed to examine the relationship of variables investigated in this study. The results (Table 4-3) showed that all variables had positive β-coefficients. Detailed discussion on the β-coefficient and their significance are described in section 4.1.4.

Furthermore, all variables in the conceptual model had significant p-values (<0.05), which means that all variables had a significant effect on purchase and consumption behaviour.

4.1.4 Analysis of factors in the conceptual model

4.1.4.1 IVM purchase and consumption behaviour

In this study, the IVM purchase variable measures the purchase intention and consumption behaviour based on consumers' willingness to try (WTT), willingness to buy (WTB), and willingness to eat (WTE). Both IVM purchase and consumption behaviour were influenced by their views on IVM and other variables (such as environment and sustainability, health and safety, cultural beliefs and current purchase and consumption behaviour). As a result, these variables had a significant and robust relationship.

Typically, most studies examine the effect of perceptions on IVM purchase and consumption, but different names (like absolute opposition (Wilks et al., 2019) and acceptance (Bryant and Dillard, 2019d; Bryant et al., 2019c)) and different aspects of purchase and consumption behaviour will be investigated (like willingness to eat (WTE) (Wilks and Phillips, 2017; Wilks et al., 2019), willingness to purchase (WTP) (Mancini and Antonioli, 2019)). Similar approach have been observed in other studies (Bryant and Dillard, 2019d; Bryant et al., 2019c; Buckingham et al., 2003; Gómez-Luciano et al., 2019; Mancini and Antonioli, 2019; Wilks and Phillips, 2017). Findings from the studies referenced are discussed in detail in the paragraph below.

Table 4-4 Means of survey questions on behavioural intentions towards IVM purchase and consumption

	Question number	Question	Mean	SD	5-point Scale
Willingness to try (WTT)	Q6.9	Do you think you would try in-vitro meat (IVM) for its nutritional profile?	3.42	±1.05	(Strongly disagree – Strongly agree)
	Q6.18	Do you think you will try it over traditional meat?	3.31	±1.05	(Strongly disagree – Strongly agree)
Willingness to buy/ purchase (WTB / WTP)	Q8.2	Do you think you would buy in-vitro meat (IVM) over traditional meat?	3.35	±0.98	(Definitely not– Definitely yes)
	Q8.3	Do you think you would buy in-vitro meat (IVM) if it is affordable	3.53	±1.08	(Strongly disagree – Strongly agree)
	Q8.6	Do you think you would buy in-vitro meat (IVM) regularly	2.97	±1.04	(Definitely not to Definitely yes)
		Do you think you would buy in-vitro meat (IVM) if it is labelled as guilt-free meat?	3.64	±1.20	
	Q8.4	Do you think you would buy in-vitro meat (IVM) if it would be cheaper than conventional meat?	3.4	±0.97	Strongly disagree-Strongly agree
	Q8.5	Do you think you would buy in-vitro meat (IVM) over conventional meat even though it is expensive?	2.4	±1.04	

Results (Table 4-4) showed that 37.37% of New Zealanders were neutral about trying IVM over traditional meat (n=206, mean=3.31, SD=±1.05) even though IVM had a health-friendly nutrition profile (n=206, mean=3.42, SD=±1.05). In addition, 39.32% of New Zealand consumers were neither willing nor unwilling about buying IVM over traditional meat (n=206, mean=3.35, SD=±0.98). As seen in Table 4-4, the means for most questions were close to 3 on a 5-point scale, which implies that consumers had neutral opinions. Neutrality in opinion may be due to the nascent IVM technology itself. Since the technology is relatively new and unknown to consumers, neutral opinions are given.

Results showed that 47.57% of NZ consumers would not buy IVM regularly (n=206, mean=2.97, SD=±1.04) compared to traditional meat. Furthermore, 35.43% of NZ consumers would not buy IVM (n=206, mean=2.4, SD=±1.04) compared to the highly priced traditional meat. From the results in Table 4-4, it is evident that New Zealand consumers have minimal interest in engaging with IVM, and this could be due to – cultural differences in diet, due to lack of exposure/familiarity with IVM, due to novelty of technology involved and lastly due poor acceptance observed among consumers in this study. New Zealand consumers are one of the highest meat consumers in the world (Pereltsvaig, 2013; The Economist, 2012). As a result, their diet is mainly meat-based and, in such cases, there will be higher meat attachment and reduced inclination to meat alternatives. Thus, New Zealanders were less interested in trying or buying IVM.

Most New Zealand consumers (36.40%) were unaware of IVM and only 23.78% were slightly aware of IVM (n=206, mean=2.27, SD= 1.24) prior to the study, indicating that the familiarity among consumers was very low. Familiarity is created when there is media exposure on IVM technology. Unfortunately, IVM awareness in NZ is not as high compared to European (UK, Belgium, France, Italy and Netherland) and Western countries (USA and Canada). Thus, New Zealanders were less interested in trying or buying IVM. Due to NZ consumers cultural palate, poor media exposure and technological infancy, the perception of IVM was in general neutral and not as strong as consumers in the Europe or Western countries. This may account for poor IVM purchase and consumption intentions amongst NZ consumers.

Mancini et al. (2019) study, showed that perceptions on IVM improved when consumers (n=525) were provided with more information on IVM, and as a result, even the purchase and consumption behaviour (willingness to try-WTT=54% and willingness to buy-WTB= 44.2%) increased. Likewise, Bryant et al (2019), showed that consumer perceptions affected consumption and behavioural intentions of consumers (n=480), (WTT=64% probably or definitely willing to try WTB IVM regularly = 49.1% probably or definitely willing to buy). Similarly, results from Wilks et al. (2017) study showed that consumer perceptions influenced IVM purchase and consumption, as consumers (n=673) with perceptions in favour of IVM had higher consumption likelihood (mean WTT IVM= 2.3, mean WTE IVM= 2.94).

Hocquette et al (2015) study further showed that consumer perceptions often influenced purchase and consumption intentions, as consumers (42%, n=817) who believed that IVM would not reduce the negative impacts of livestock industry had reduced intentions of IVM consumption (only 5.3% of the French consumers that would eat IVM). Similarly, Slade et al (2018) showed that Canadian consumers (n=533) perceived IVM unfavourably (due to taste and health risks) and showed disclination towards IVM purchase and consumption (mean=0.126, on a 5-point scale).

4.1.4.2 Environment and sustainability

Environmental attitude towards IVM depends on factors related to general environmental and animal welfare awareness, as well as consumers' openness towards sustainable alternatives.

The overall effect of environmental and sustainability variable on IVM purchase was significant (p=0.036, Mean=3.18, SD=±1.18, KMO=0.854, Cronbach's alpha=0.861) (Table 4-2). There was a positive correlation between the variables (β=0.100). In other words, a one unit increase in consumers' perceptions on environment and sustainability will lead to increase in IVM purchase intentions among consumers. Hence, the environment and sustainability variable was a significant factor that influenced IVM purchase intentions in this study. The means for questions under the environment and sustainability variable (Table 4-5) showed varied results. It is evident from the results that although 46.11% of NZ consumers are environmentally conscious (n=206, mean=3.83, SD=±1.01) and 38.83% are pro-animal welfare (n=206, mean=3.84, SD=±1.11), not many consumers are aware of the negative repercussions of the traditional meat industry (n=206, mean=2.94, SD=±1.29). As a result, most consumers do not understand the importance of sustainable alternatives and have decreased purchase intentions towards IVM, as seen in Table 4-5.

Table 4-5 Means of survey questions on environmental and animal welfare awareness that indirectly influences consumer perceptions of IVM.

Blocks	Question numbers	Questions	Mean	SD	Scale
Environment and Sustainability	Q4.1	How important is the environment to you?	3.83	±1.01	Not at all important – Extremely important
	Q4.2	Are you aware of the negative environmental effects of the conventional meat industry?	2.94	±1.29	Not at all aware- Extremely aware
	Q4.3	Do you think that the traditional meat industry contributes to global issues such as greenhouse gas emission and changes in climate?	3.62	±1.14	Strongly disagree – Strongly agree
	Q4.4	Are you aware of the fact that the conventional meat industry has a higher carbon footprint compared to other meat alternatives?	2.99	±1.31	Not at all aware – Extremely aware
	Q4.5	Are you aware of the terms such as sustainability and a sustainable environment?	3.46	±1.22	
	Q4.9	Do you believe in animal welfare?	3.84	±1.11	Strongly disagree – strongly agree
	Q4.6	Are you familiar of sustainable meat alternatives?	2.64	±1.14	Not at all familiar – Extremely familiar
	Q4.7	Do you think you are open to technologies related to the food industry?	3.59	±1.11	Not at all open to extremely open
	Q4.8	Are you familiar with meat analogues such as plant-based	2.66	±1.19	Not at all familiar – Extremely familiar

		protein, insect protein, in-vitro meat (IVM), vegan fish and fishless seafood?			
	Q4.10	Are you familiar with technologies such as cellular agriculture / In-vitro meat (IVM) technology and tissue engineering?	2.21	±1.18	

On the contrary, Wilks et al. (2017) showed that US consumers (n=673, mean =1.97, 1- much more – 5 much less) who were environmentally conscious agreed that IVM was an environmentally friendly alternative, and subsequently resulted higher purchase and consumption intentions. Similarly, Mancini et al. (2019), showed that environmentally conscious Italian consumers (n=525) perceived IVM favourably that resulted in higher purchase intentions (44.2% said yes to willingness to buy IVM).

Furthermore, this variable also measures consumer's general awareness and their willingness towards sustainable alternative consumption, such as plant-based meat and cell-based meats. The underlying question here was to check for food neophobia among consumers towards novel food products (Bryant et al., 2019c; Cox and Evans, 2008; Flight et al., 2003; Wilks et al., 2019). The means of questions in Table 4-6 showed that New Zealand consumers are not entirely open to sustainable alternatives. A study on US consumers (Scott et al., 2016; Wilks et al., 2019) reported that consumers had moral absolutism[20] and disgust towards genetically modified (GM) foods. In addition, moral absolutism was found to be more evident among US consumers compared to European consumers (Scott et al., 2016). The reduced openness to sustainable alternatives in this study could also be linked to subtle absolutism. Furthermore, reduced openness towards sustainable alternatives can also be due to eating behaviour. For example, consumers with high meat consumption have minimal interest in engaging with sustainable alternatives (Tucker, 2014), as is the case with New Zealand consumers. Besides, openness to sustainable alternatives, consumer reluctance towards IVM is also due to other factors like unnaturalness (Wilks and

[20] **Moral absolutism** is an **ethical** view that all actions are intrinsically right or wrong. Stealing, for instance, might be considered to be always immoral, even if done for the well-being of others (Source: Wikipedia)

Phillips, 2017), disgust, fear of perceived risks (Verbeke et al., 2015) and food neophobia (Bryant et al., 2019c; Wilks et al., 2019).

The results in Table 4-5 showed that consumers were not familiar of sustainable alternatives available in the market. In addition, NZ consumers were less familiar with IVM, cell-based meats, and other plant-based protein (refer Table 4-6). Similarly, only 13% (n=180) of Belgium consumers (Verbeke et al., 2015b) and 14% (n=1296) Netherlands consumers were familiar with IVM (Flycatcher, 2013). In addition, the majority of US consumers (57.3%), Chinese consumers (35.5%) and Indian consumers (25.5%) were not at all familiar with IVM (Bryant et al., 2019c). Generally, lack of familiarity with IVM among consumers is one of the primary concerns for poor purchase intentions as previously explained in section 2.2.1.3.6.

4.1.4.3 Health and safety

Consumer opinions on the health and safety aspects of IVM mainly focuses on food safety and perceived health concerns, such as cancer and other food-related diseases. These opinions collectively form the health and safety variable, which indirectly affects IVM purchase intentions.

In our study, health and safety variables are vital as it measures consumer concerns, which influences consumer perceptions of IVM. Consumers are wary about the perceived health and safety risks of IVM (Verbeke et al., 2015) due to unnaturalness, scientific distrust ad unfamiliarity (Verbeke et al., 2015).

Table 4-6 Means of survey questions on health and safety profile of IVM that indirectly influence IVM purchase and consumption behaviour

Block	Question numbers	Questions	Mean	SD	Scale
Health and Safety	Q9.7	Do you think in-vitro meat (IVM) should be produced by government-approved agencies?	2.43	±0.99	Definitely not – definitely yes
	Q10.1	Do you think in-vitro meat (IVM) has any health and safety concerns?	3.27	±0.94	Strongly disagree – strongly agree
	Q10.2	Do you think in-vitro meat (IVM) is likely to cause any disease?	2.47	±1.01	Not at all likely – Extremely likely

Block	Question numbers	Questions	Mean	SD	Scale
	Q10.3	Do you think in-vitro meat (IVM) is cancerous as it involves stem cells?	2.86	±0.89	Strongly disagree – strongly agree
	Q10.4	Do you think in-vitro meat (IVM) will have any food safety risk?	2.54	±0.88	Definitely not – Definitely yes

Results in Table 4-6 showed that IVM health and safety (M=2.72, SD=± 0.95, KMO=0.714, Cronbach's alpha=0.573) had a significant effect on IVM purchase and consumption behaviour (p=0.001). There was a positive correlation (β =0.316) between the factors, which suggest that perceived health and safety concerns on IVM are likely to influence IVM purchase intentions. Findings on IVM health and safety were also observed in other studies (Bryant et al., 2019a; Gómez-Luciano et al., 2019; Mancini and Antonioli, 2019; Tucker, 2014; Wilks and Phillips, 2017) Some studies showed that consumer perceptions on health and safety of IVM, which are not in favour of IVM lead to decreased purchase intentions (Hocquette et al., 2015; Tucker, 2014). For example, Hocquette et al (2015) study showed that 41.2% of French consumers believed that IVM would not be healthy (n=865) and this greatly reduced IVM purchase intentions.

Results in Table 4-6 showed that 33.49% of New Zealand consumers would probably not buy IVM (n=206, mean=2.43, SD=±0.99) and 37.37% were neutral about buying IVM, even if it was manufactured by government-approved agencies, over the multinational companies or start-ups. Furthermore, 30.09% of New Zealand consumers had health and safety concerns about IVM, whereas 44.6% of consumers were neutral about IVM health and safety (n=206, mean=3.27, SD=±0.94). On the other hand, 34.46% of NZ consumers perceived IVM as probably having food safety risk and 41.26% of consumer had neutral opinions on IVM food safety risk (n=206, mean= 2.54, SD=±0.88). These findings could be due to the nature of IVM, as it is an artificial/man-made food product using animal cells. Health and safety concerns was one of the common reasons for rejection towards IVM, this concern is widely seen in IVM literature (Bryant and Dillard, 2019d; Gómez-Luciano et al., 2019; Hocquette, 2016; Mancini and Antonioli, 2019; Verbeke et al., 2015; Wilks and Phillips, 2017). Health and safety concerns observed in the literature was briefly described under perceived risks sections (2.2.1.3.5). These concerns are due to the nature of the product (IVM), as it is genetically modified using tissue engineering. Due to the involvement of genetic modification, IVM is susceptible to disgust, concerns on unnaturalness and fear of perceived risks (Verbeke et al., 2015). In addition, some studies showed that consumers were worried about mutations, cancer arising from IVM (Verbeke et al., 2015). However, New Zealand consumers did not believe in cancer or mutations occurrence in IVM technology.

4.1.4.4 Cultural beliefs

The cultural beliefs variable measures the IVM purchase intention based on the consumers cultural identity and beliefs. The underlying question here was to check for cultural implications on IVM as a sustainable meat alternative.

The effect of cultural beliefs (mean=2.05, SD=±1.08, Cronbach's alpha=0.622, KMO=0.749) on IVM purchase and consumption behaviour was significant ($p<0.001$). There was a positive correlation between the variables (β=0.516), which suggest that cultural beliefs are likely to influence IVM purchase intentions.

Table 4-7 Means of survey questions on consumer's cultural beliefs that indirectly influence on IVM purchase and consumption behaviour.

Block	Question number	Question	Mean	SD	Scale
Cultural beliefs	Q5.1	Do you think you would likely consume in-vitro meat (IVM) if your religious beliefs permitted?	2.4	±1.09	Definitely not - Definitely yes
	Q5.2	Do you think you would eat in-vitro meat (IVM) if the religious leaders informed you?	2.16	±1.08	Strongly disagree- strongly agree
	Q5.3	In some religions, the intellectual level of the animal to be slaughtered for meat purposes are considered, such as "only those animals with lower intellectual capacity and pain sensation are to be slaughtered" whereas, in-vitro meat (IVM) does not require any animal slaughter at all.	1.73	±1.09	Strongly disagree – strongly agree

Block	Question number	Question	Mean	SD	Scale
		Do you think in-vitro meat (IVM) is a better meat alternative than conventional meat?			
	Q5.4	Do you think you would opt for in-vitro meat (IVM) if there will be halal[21] or kosher[22] options available?	1.89	±1.05	Strongly disagree – Strongly agree

Halal - refers to what is permissible or lawful in traditional Islamic law
Kosher - Kosher foods are those that conform to the Jewish dietary regulations of kashrut (dietary law).

The results in Table 4-7 showed that 11% NZ consumers (n=206, mean=2.4, SD=±1.08) would not consume IVM, even if their religious beliefs permitted (n=206, mean=2.4, SD=±1.09), or if religious leaders promoted IVM (n=206, mean-2.16, SD=±1.08), or if IVM was available as halal or kosher meat (n=206, mean=1.89, SD=±1.05). These results showed that purchase intention towards IVM was not influenced by consumers' cultural beliefs.

4.1.4.5 Current purchase behaviour and consumption

The current purchase behaviour and consumption variable measures the IVM purchase intentions based on consumers current purchase behaviour and meat-eating behaviour. The underlying idea here is to test the effect of current eating and purchase behaviour on IVM purchase and consumption behaviour.

The effect of current purchase behaviour and consumption (mean=2.39, SD=±1.14, Cronbach's alpha=0.692, KMO=0.551) on IVM purchase and consumption behaviour was significant (p=0.034). There was a positive correlation between the variables (β=0.097), which suggest that current purchase behaviour and consumption are likely to influence IVM purchase intentions.

[21] Halal - refers to what is permissible or lawful in traditional Islamic law
[22] Kosher - Kosher foods are those that conform to the Jewish dietary regulations of kashrut (dietary law)

Table 4-8 Means of survey questions on consumers' current purchase behaviour and consumption that indirectly influence on IVM purchase and consumption behaviour.

Block	Question number	Question	Mean	SD	Scale
Current purchase behaviour and consumption *Q3.3, Q3.11 and 3.12 have cumulative Check all that applies (CATA) counts	Q3.2	How often do you eat meat?	3.69	±1.11	Never – Always (5-point scale)
	Q3.3*	Which type of meat do you prefer the most? Check all that applies	3.04	±1.14	a) Poultry b) Pork c) Beef d) Fish and other seafood e) Others
	Q3.11*	What does your meat intake look like? Check all that applies	3.49	±0.9	a) Once a month b) Once a fortnight c) Once a week d) Every meal e) Never
	Q3.12*	When do you usually eat meat? When I am ...? Check all that applies	3.52	±1.14	a) Hungry b) Bored c) Crave d) Sad e) Happy
	Q6.1	Do you think you will still buy conventional meat although it has a higher carbon footprint?	3.14	±1.18	Strongly disagree – strongly agree
	Q6.2	Do you think you would give up meat for the sake of animals?	2.78	±1.27	Strongly disagree – strongly agree
	Q6.3	Do you think you would buy plant-based products if it had a better health star rating compared to meat products?	4.08	±1.18	Definitely not – Definitely yes

Results in Table 4-8 showed that 53.88% of NZ consumers had higher meat intake (n=206, mean=3.69, SD=±1.10), with poultry (70.87%) being the most preferred choice of meat. These results showed that NZ consumers had a higher meat attachment and would not give up meat for the welfare the animals (n=206, mean=2.78, SD=±1.27). However, 31.06% of NZ consumers were uncertain about buying traditional meat due to its carbon footprint (n=206, mean=3.14, SD=±1.18) but were willing to buy plant-based meat alternatives compared to traditional meat products (29.35%, n=206, mean=4.08, SD=±1.18), mainly due to its nutritional profile. These findings demonstrated that although NZ consumers are fond of meat, they were willing to engage with plant-based meat alternatives but were reluctant to try IVM. Reasons for such hesitance among NZ consumers can be due to several reasons, such as the nature of IVM, differences in eating habits, higher meat attachment and general food neophobia as previously discussed in section 2.2.1.2. Nevertheless, if NZ consumers current purchase and consumption behaviour changes, then it would result in higher IVM purchase intentions among consumers.

4.2 Preliminary cell culture studies

Preliminary cell culture studies were carried out on culture murine myoblast C2C12 cells on 3D-printed hydrogel scaffolds. The cell culture study was merely a trial and not a primary objective. This preliminary cell culture trials would be beneficial for future research on in-vitro meat (IVM) production. In-vitro meat (IVM) cultured on gelatin methacrylate (gelMA) scaffold can be harvested, once the muscle fibres are fully developed. Murine myoblast C2C12 cells are most suited for IVM purposes due to its excellent regeneration capacity, multipotency and myogenesis ability. Skeletal muscle cells may not be suitable for cultivation of IVM, as it is adult stem cells with reduced regeneration capacity

4.2.1 Microscopy of GelMA scaffolds

4.2.1.1 8% gelMA

The 8% gelMA 2-layers when imaged immediately after seeding, appeared to be a suitable scaffold for cell culturing. However, there was no cell attachment or entrapment at the seeding point (appendix 1). The cells at seeding point imaged 3hrs after seeding, appeared to be healthy and growing well. Some of the cells are evident at the seeding point (inside the pores of scaffold marked in red), while most of the cells migrated towards the scaffold matrix, as seen in Figure 4-1.

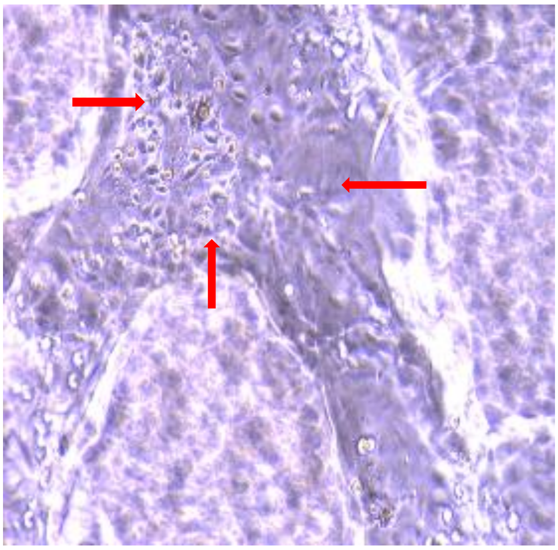

Figure 4-1 C2C12 cells in 8% gelMA 2-layers scaffold, imaged after 3 hours of cell seeding.

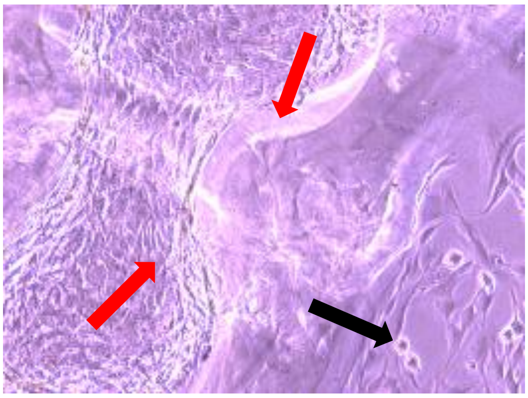

Figure 4-2 C2C12 cells in 8% gelMA scaffolds 2-layers imaged after 35 hours of seeding.

After 35hrs of seeding, the scaffold appeared to be a suitable scaffold for cell culturing, as it facilitated the growth and attachment of cells (Figure 4-2). Some cells aggregated forming micropattern -like structures in the scaffold (marked in red), which can promote the elongation of cells and muscle formation. Hypothetically, if C2C12 cells can be grown on these scaffolds, it is possible to even grow skeletal muscle cells on gelMA scaffolds to produce edible in-vitro meat. However, dead and/or unattached cells can be seen in the vicinity (marked in black).

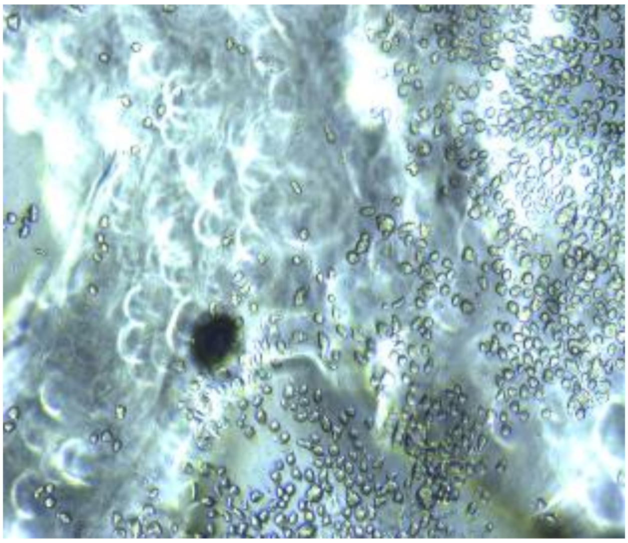

Figure 4-3 C2C12 cells in the pores of 8% gelMA 4-layers scaffold, imaged immediately after seeding.

The 8% gelMA – 4 layers scaffold imaged immediately after seeding appeared to have entrapped cells well inside the scaffold, and the cells did not get washed away (Figure 4-3) when media was added, unlike with the 2-layers scaffold. This is likely due to the larger pores in the 4-layers scaffold, which can hold more cells, as opposed to 2-layers scaffold.

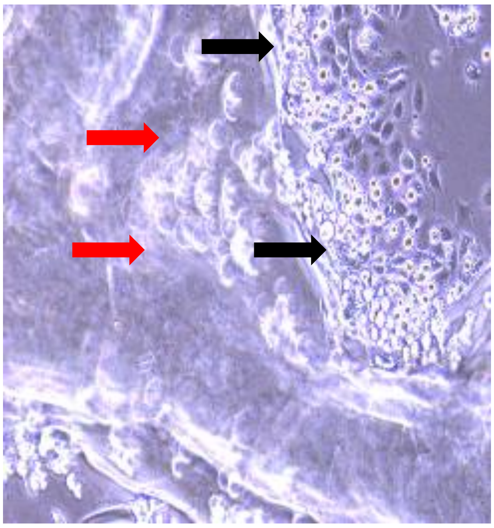

Figure 4-4 C2C12 cells in 8% gelMA scaffold 4-layers, imaged after 3 hours of seeding.

After hours of seeding (Figure 4-4), some of the cells (shown in red) were observed on the surface of the scaffold, but the majority of cells (shown in black) were found in the well plate (not pictured). Reasons for finding cells in the well plate is due to cell detachment, which is caused by contamination, incubator temperature fluctuations, inappropriate environmental gas mixture and inappropriate /insufficient cell culture substrate/surface (Sigma Aldrich, 2019)

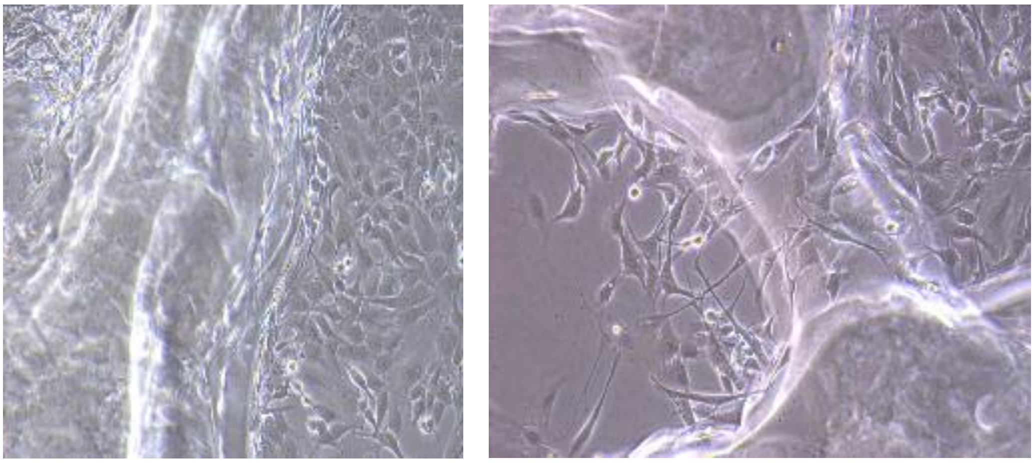

Figure 4-5 C2C12 cells in 8% gelMA scaffold-4 layers, imaged at 13 hours after seeding.

Cells imaged after 13hrs of seeding appeared to be healthy and well attached as seen in Figure 4-5. Some cells were found growing along with the scaffold matrix, with the rest were found inside the pores of the scaffold. However, at the 35th hour of seeding, fungal contamination was evident. Contamination could be due to use of non-sterile surfaces and equipment's, spillage, incorrect handling of pipette and bottles, due to sedimentary dust or particles of skin and working coughing or sneezing(Sigma Aldrich, 2019). Overall, although the scaffold was suitable for cell culture, cell attachment needs to be improved and contamination prevented. Cell detachment can be controlled by adding calcium, ceruloplasmin or citrate. Whereas, contamination can be managed by strict adherence to aseptic techniques (Sigma Aldrich, 2019)

4.2.2 Microscopy of GelMA and collagen scaffolds

4.2.2.1 8% gelMA +1% collagen

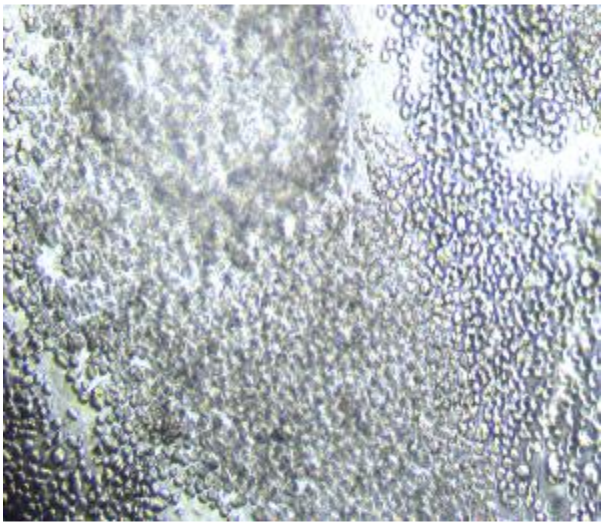

Figure 4-6 C2C12 cells in 8% gelMA and 1% collagen scaffold - 2 layer, imaged immediately after seeding.

The cells in 8% gelMA and 1% collagen 2-layer scaffolds can be seen in the seeding point, (Figure 4-6). It can be observed that cells were attached to the scaffold without being washed away by media replacement. This scaffold exhibited excellent biocompatibility with cells and was thereby suitable combination to culture c2c12 cells. When cells were imaged 3 hours after seeding, cell migration from inside the scaffold pores towards the scaffold matrix was evident as seen in Figure 4-7. Cellular movement is an indication of biocompatibility between C2C12 cells and the scaffold, that may facilitate further growth of cells. Hence there is a possibility of growing skeletal muscle cells on a gelMA and collagen scaffold to harvest edible meat in future.

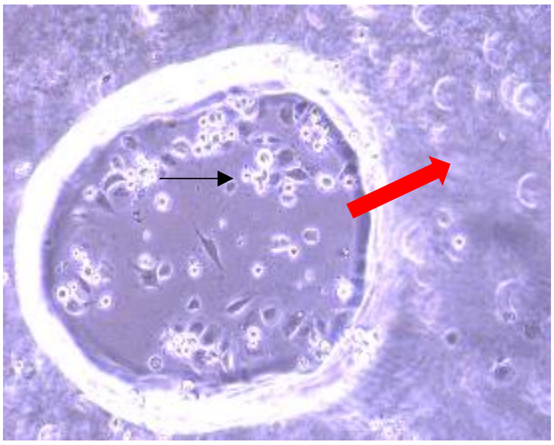

Figure 4-7 C2C12 cells in 8% gelMA and 1% collagen scaffold-2 layers, imaged after 3 hours of cell seeding (Marking: direction of migration in red, cells in black).

The 2-layer scaffold observed after the 3rd hour of seeding was similar to the image obtained immediately after seeding (Figure 4-7). Some cells, in another focal point, appeared to form elongated fibre-like structures. However, it was difficult to confirm if the fibre-like structure (marked in black) were muscle fibres, or if it were cells growing along the scaffold curvature. When the scaffolds were observed after the 13 hours of seeding, there was increased cell movement and elongation as seen in Figure 4-8. On the other hand, scaffolds at the 35th hour of seeding, dense cell growth was observed at the seeding point (pore), due to lack of space inside the pores (seen in red). Consequently, some cells migrated into the scaffold and moved towards the edge of the scaffold, as seen in Figure 4-9.

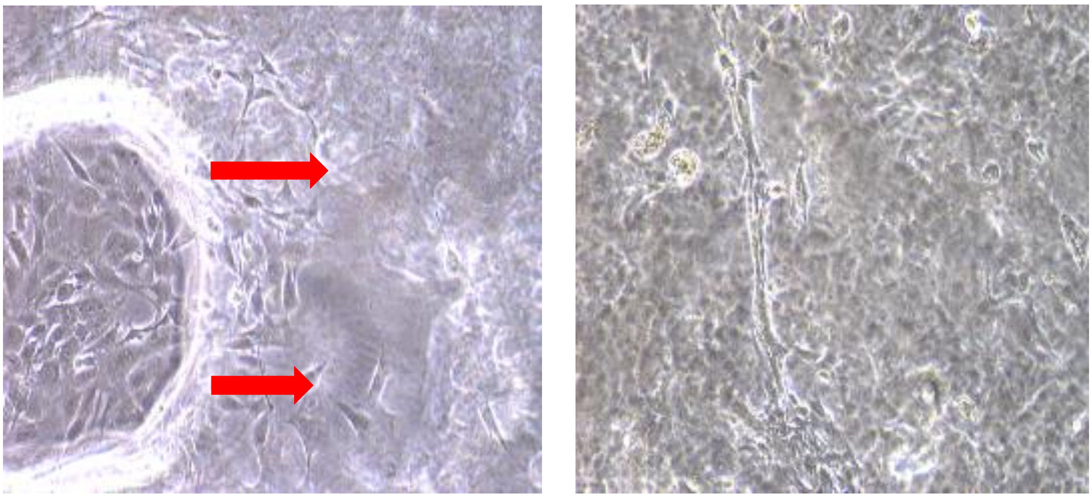

Figure 4-8 C2C12 cells in 8% gelMA and 1% collagen scaffold - 2 layers, observed after 13th hour of seeding.

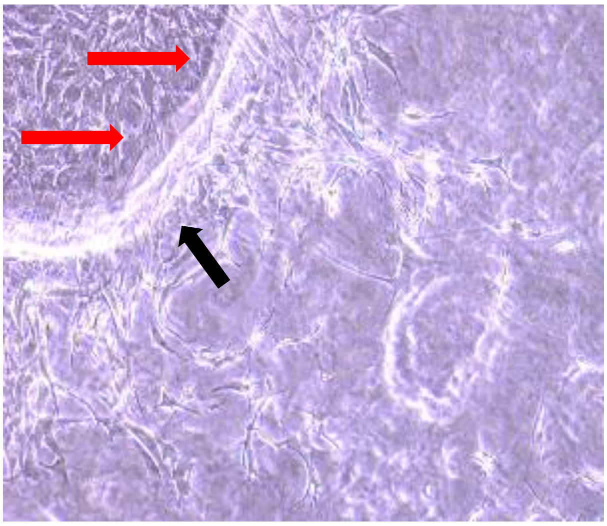

Figure 4-9 C2C12 cells in 8% gelMA and 1% collagen scaffold – 2 layers, imaged after 35th hour of seeding.

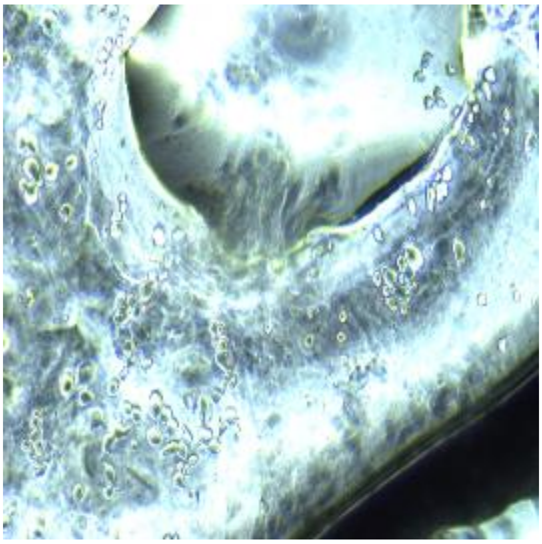

Figure 4-10 C2C12 cells in 8% gelMA and 1% collagen scaffold - 4 layers, imaged immediately after seeding.

The 4-layer scaffold with 8% gelMA and 1% collagen imaged immediately after seeding (Figure 4-10) showed cell entrapment without cells being washed away when media was added, unlike the 2-layer scaffold. This is likely due to the larger pores in the 4-layer scaffold, which can hold more cells, as opposed to the 2-layers scaffold. Furthermore, when these scaffolds were imaged at 3hours after seeding, cells appeared to be concentrated in the scaffold pores, but not in the scaffold matrix, as seen in Figure 4-11. Yet again, it is due to the cellular movement towards pores in the scaffold. After the 13th hour of seeding, cell migration and elongation were observed similar to the 2-layer scaffold of the same composition as seen in Figure 4-12. These scaffolds when observed at the 35th hour after seeding however had fungal contamination as seen in Figure 4-13.

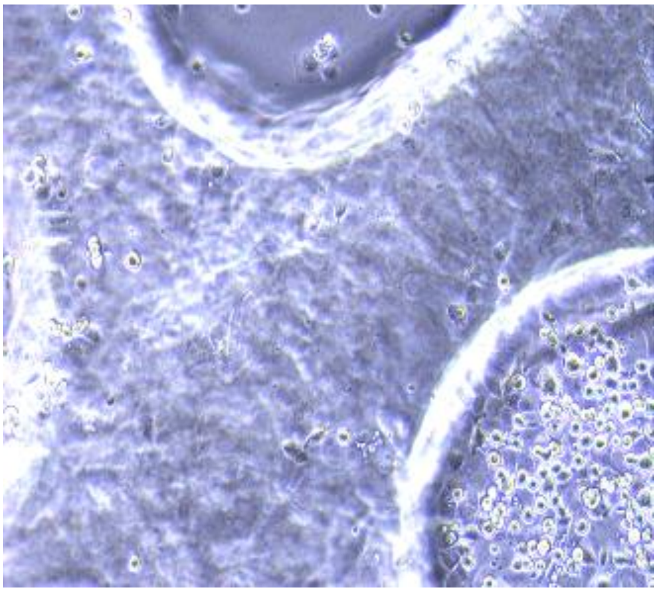

Figure 4-11 C2C12 cells in 8% gelMA and 1% collagen 4-layers scaffold, imaged after 3 hours of cell seeding.

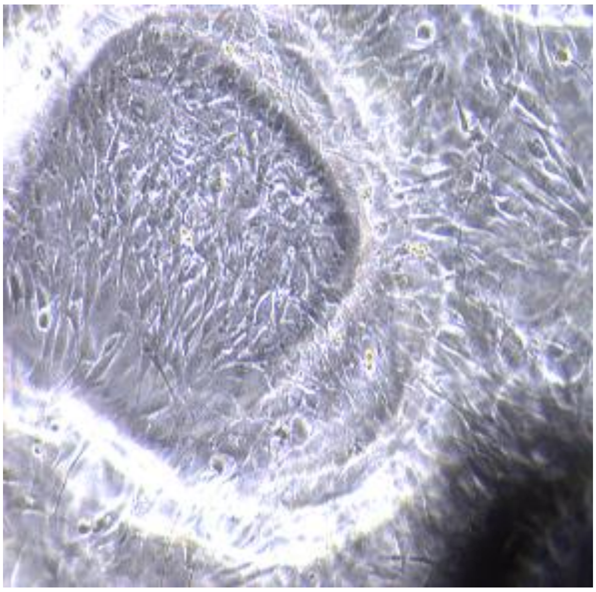

Figure 4-12 C2C12 cells 8% gelMA and 1% collagen scaffold – 4 layers, imaged after 13th hour of seeding.

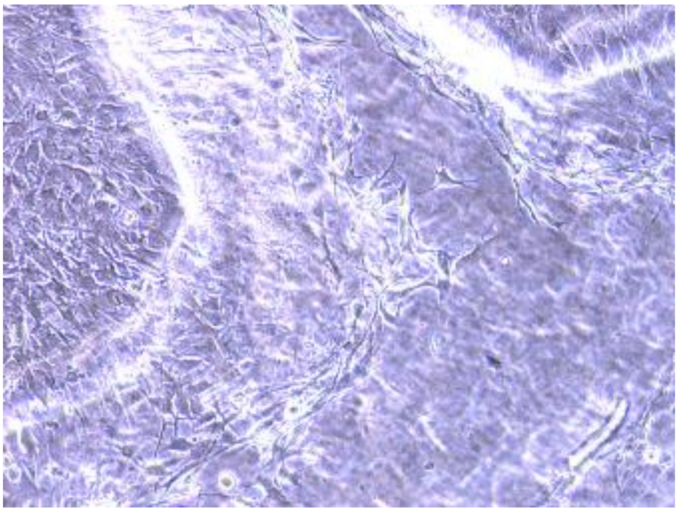

Figure 4-13 C2C12 cells in 8% gelMA and 1% collagen scaffolds – 4 layers, imaged after 35 hours of seeding.

4.2.3 Microscopy of gelMA and hyaluronic acid scaffolds

4.2.3.1 8% GelMA +1% hyaluronic acid (HA) scaffolds

All 8% gelMA and 1% hyaluronic acid scaffolds lost their form, disintegrating and forming debris (refer appendix 1 for photos) due to unclear reasons. The 2-layer scaffolds imaged immediately after seeding showed cells being washed out with media replenishment. Such cell behaviours may be due to poor cell attachment, reduced pore size of the scaffold and the incompatibility of hydrogels and cells involved. In addition, such findings could be linked to the physicochemical properties of hyaluronic acids, such as swelling and degradation properties. In some cases, the gelMA and hyaluronic acid scaffolds can get degraded when added into PBS (Poldervaart et al., 2017), this may have been the reasons for the disintegration of the scaffold.

The 4-layer scaffold also had similar problems of scaffold disintegration (refer appendix 1 for images), like other HA containing scaffolds.

The 8-layer scaffold of the same composition was unsuitable for imaging as it was too thick and had poor visibility due to cloudiness. Yet, some cells and cell aggregates were spotted in the seeding point vicinity. However, the images of cell-laden scaffold s were unfavourable as it was not clear.

4.2.4 Pipette scaffolds

4.2.4.1 8% gelMA pipette scaffold

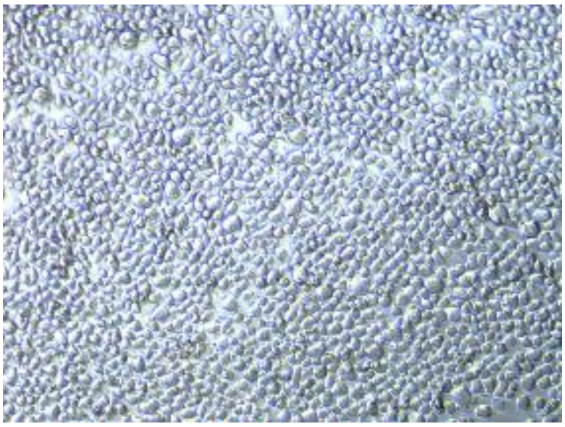

Figure 4-14 C2C12 cells in the pores of 8% gelMA pipetted scaffold, imaged immediately after seeding.

Pipetted scaffold with 8% gelMA, when imaged immediately after seeding, showed that the cells were intact in the scaffold, as seen in Figure 4-14. These pipetted scaffolds had no problems with cell detachment and washing away, unlike the 3D printed mesh scaffolds. Like other scaffolds, these scaffolds also showed cellular processes like elongation and movements after the 13th hour of seeding as seen in Figure 4-15. However, even these scaffolds were affected by fungal contamination (marked in red) at the 35th hour of seeding as seen in Figure 4-16

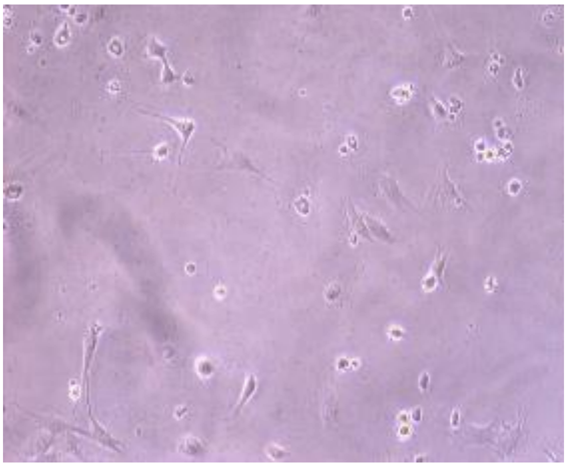

Figure 4-15 Cells are healthy in 8% gelMA pipetted scaffolds observed at the 13th hour after seeding.

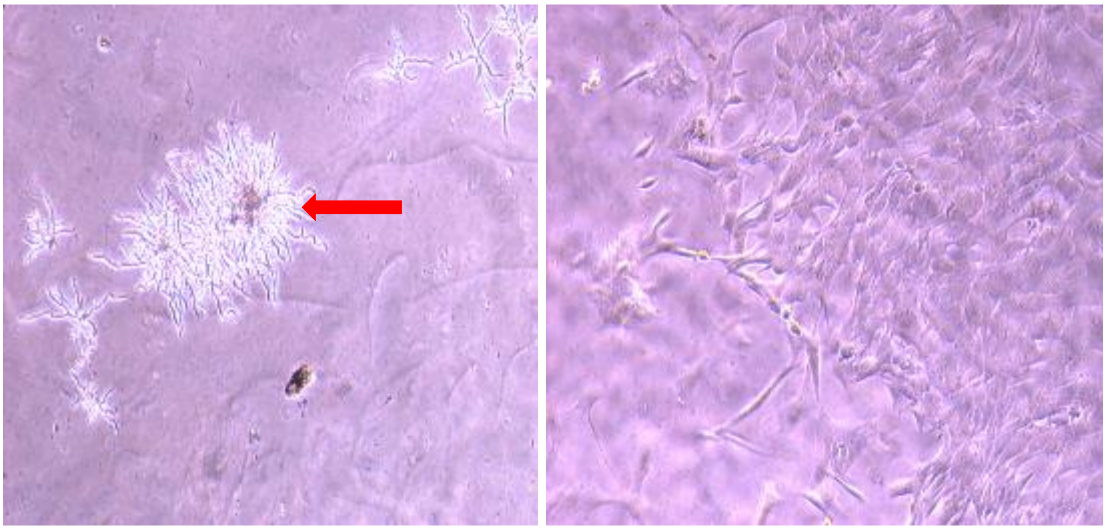

Figure 4-16 C2C12 cells in 8% gelMA pipetted scaffolds, imaged after 35 hours of seeding showing fungal contaminations (marked in red).

There were no pipetted scaffolds of 8% gelMA and 1% collagen, as the scaffolds were damaged on reception. However, there were several other gelMA mesh scaffolds to perform cell culture studies.

4.2.4.2 8% gelMA and 1% HA pipette scaffold

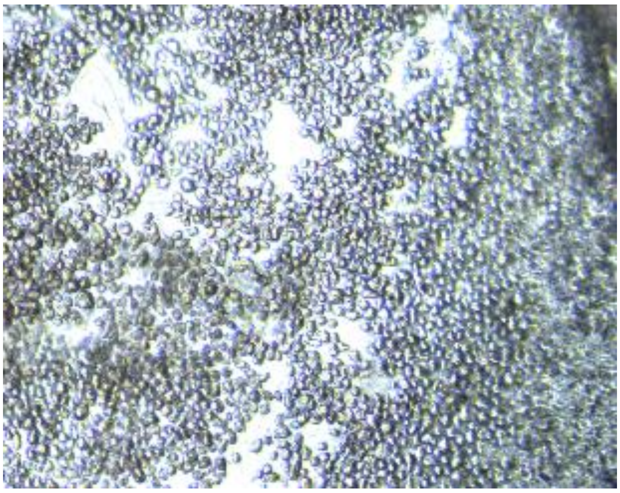

Figure 4-17 C2C12 cells in 8% gelMA and 1% hyaluronic acid (HA) pipetted scaffolds, imaged immediately after cell seeding.

The pipette scaffold with 8% gelMA and 1% HA imaged immediately after cell seeding showed well-distributed cells and attached, as seen in Figure 4-17 The pipetted scaffolds dissolved when viewed after the 3rd hour of cell seeding. The dissolvement of scaffold could be linked to human error during the preparation of hydrogels, such as miscalculation of hydrogel constituents based on their molecular weight. However, the well plate had some scaffold residue stuck at the bottom, and these residues facilitated traces of cell growth at the 13th hour of seeding, as seen in Figure 4-18.

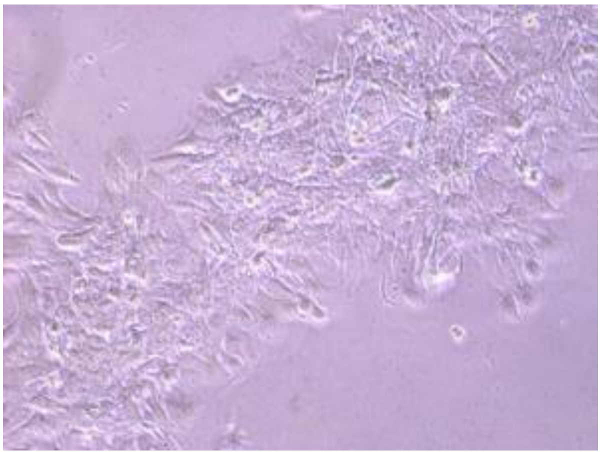

Figure 4-18 C2C12 cells on 8% gelMA and 1% HA pipetted scaffold residues, imaged at 13hours after seeding.

4.2.4.3 Problems encountered during cell culture studies of cell-laden gelMA and other scaffolds.

All the 8-layer scaffolds, including gelMA, were unsuitable for cell culture studies since they were too thick and dense to perform cell micrography. Additionally, scaffolds turned out to be cloudy that prevented observation of cell behaviour and movements.

Other problems concerning use of these scaffolds are their inability to hold onto cells as they were washed away on the addition of media. In addition, some scaffolds ended up being detached and were found afloat in the well plate with the addition of media. At this stage of research, more work is required on hydrogel formulation, biocompatibility and physicochemical testing of hydrogels.

Scaffolds containing hyaluronic acid tends to absorb the media when replenished due to their hydrophilic nature. The ability of these scaffolds to absorb water also makes them more susceptible to breakage. The most challenging limitation of using scaffolds was their susceptibility to fungal contamination, despite the use of antifungals and antibacterial in the study. However, it is suspected the contamination is likely due to poor usage of aseptic techniques

Other planned protocols such as trypan blue and Alamar blue assay could not be performed in this study due to contamination of scaffolds, that required disposal. Due to loss of scaffolds, microscopy of C2C12 cell-laden scaffolds during cell proliferation and differentiation could not be carried out. However, in Appendix 1 images of C2C12 cell proliferation and differentiation stages without any scaffold (control) are shown.

5. Chapter 5. CONCLUSIONS

This study sets out to understand the perceptions of IVM by New Zealand consumers, particularly with respect to IVM purchase and consumption behaviour. The general results indicated that if the overall perception of IVM improved, then the purchase likelihood and consumption behaviour would improve as well. Although perception plays an important role in purchase intention, other variables such as consumers' current purchase and consumption behaviour, environment and sustainability awareness and consumers opinions on health and safety of IVM also influences consumers' purchase intentions. However, the results also revealed that consumers' cultural beliefs had minimal influence on IVM purchase likelihood.

Results in this study indicated that most New Zealand consumer have minimal awareness of IVM, due to which consumers mostly had neutral opinions in terms of engaging with IVM. In addition, New Zealand consumers are hesitant to engage with IVM due to lack of familiarity and thus NZ consumers need to be educated about IVM and its potential benefits. Awareness of IVM can bring about a change in its perception and in some cases even lead to consumer acceptance.

These findings add to a growing body of literature on the perception of IVM among New Zealanders. However, there may be some possible limitations in this study. The first limitation of this study is the use of convenience sampling, which may have resulted in partly biased findings. Thus, it would be beneficial to understand the perception of the general population in future studies. Furthermore, this study did not test consumers for food neophobia. Further work can investigate whether consumer reluctance towards IVM is due to food neophobia or if it is merely due to extensive meat intake.

Interestingly, findings from preliminary cell culture studies show that C2C12 cells can be grown on hydrogel scaffolds, with good cell growth observed in gelMA and gelMA+ collagen scaffolds. However, some cell-laden scaffolds were susceptible to fungal contaminations, despite the use of antifungal agents. As with the majority of studies, the methodology of cell culture studies has a number of limitations. The first is the failure to perform planned protocols, such as cell viability assay on 3D printed gelMA scaffolds (mesh), due to fungal contaminations. Thus, it would be useful if future research carried out cell viability assay with C2C12 cells and gelMA scaffold. Besides that, the cellular process such as proliferation and differentiation on mesh scaffolds could not be performed either due to loss of scaffolds. Hence, the behaviour of C2C2 cells, especially in cell proliferation and differentiation stages, on gelMA scaffold needs to be further investigated. Furthermore, a major limitation in cell culture study is contamination due to many reasons, hence it will be great to see future research on IVM production using C2C12 cells and 3D printing technology. Some limitations in this study with hydrogel formulation and 3D printing technology, included cell detachment and disintegration of scaffolds and formation of impenetrable scaffolds. Hence it would be useful, if future works are further carried out in cell culture studies utilising scaffolds that are more biocompatible and made up of penetrable hydrogels.

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APPENDIX 1

scaffold

material	pipette	2 layer	4layer	8layer
8%GelMa	3	3	3	3
8%GelMa+1%collagen	0	3	2	3
8%GelMa+1%Hyaluronic acid(HA)	3	3	3	3

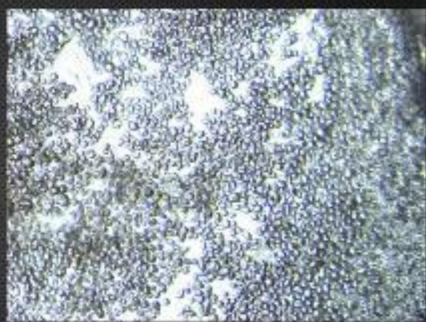
Some scaffolds were floating and not attached to the well plates. – different layer and composition

CELLS ARE BEING SELECTIVE, THEY prefer MORE ON THE WELL PLATE RATHER THAN SCAFFOLD – reason may be as the nutrient media is more in the well?

Threw away all scaffolds at 35hrs due to contamination. Could not perform differentiation.

Seeding 0h pipette

D 8%GelMa

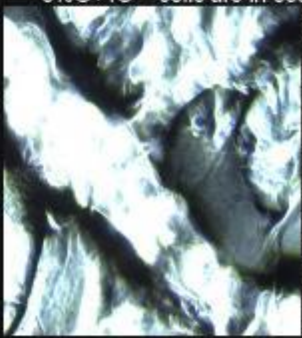


D 8%GelMa+1%Hyaluronic acid(HA)

Cells are seeded- they look normal, nothing wrong – both 8%GelMA and 1% HA+GelMA

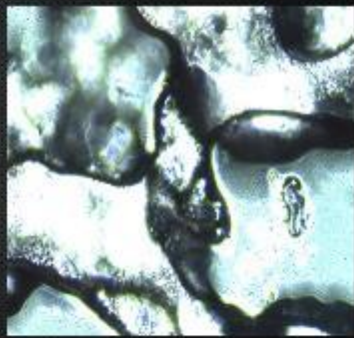
Seeding 0h 2 layer

Immediately = 2 layer- 8% GelMA and 8%G+1%HA, cells are present in the vicinity but not exactly at the point of seeding as it is washed away by the excessive addition of media.
8%G+1C = cells are in seeded point



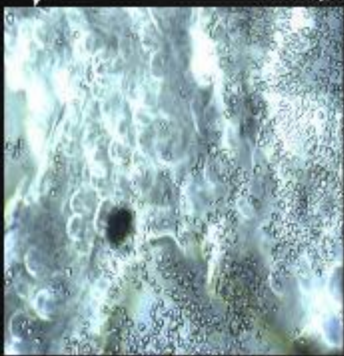
D 8%GelMa

D 8%GelMa+1%collagen

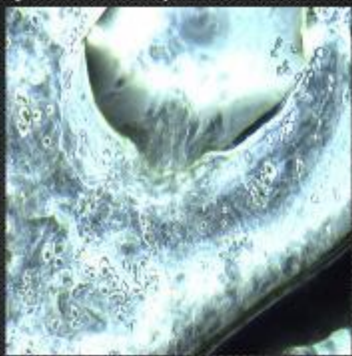


D 8%GelMa+1%Hyaluronic acid(HA)

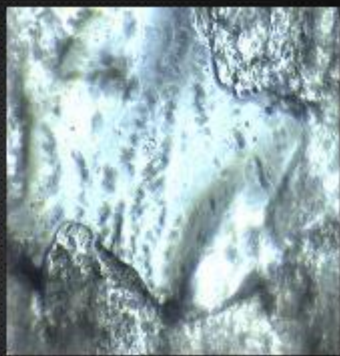
Seeding 0h 4 layer – Check for pore size with Ghada



D 8%GelMa



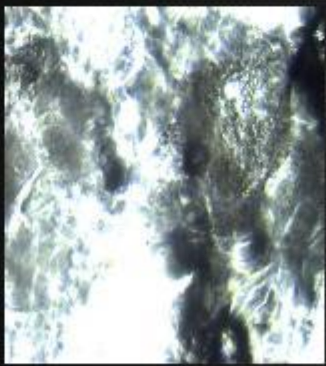
8%GelMa+1%collagen



8%GelMa+1%Hyaluronic acid(HA)

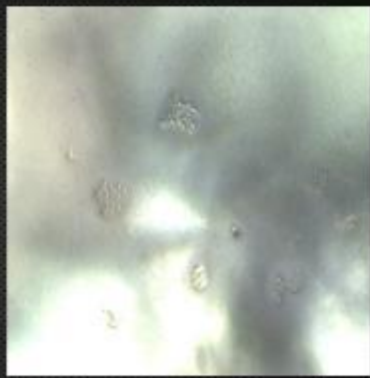
4 layer bigger than 2 layers, so the pores are bigger as well, this facilitates the cells to stay inside the scaffold, despite adding the medium – both 8%G and 8%G+1%C
But 8%G+1%HA – lost structure and disintegrated to give out debris

Seeding 0h 8 layer



8%GelMa

8%GelMa+1%collagen



8%GelMa+1%Hyaluronic acid(HA)

HA- scaffold was not transparent –yellowish – can see cell aggregates
8%G – too thick to look through
8%G+1%C = some cells on top of the scaffold

Seeding 0h Problem we met during seeding

- Scaffold with HA absorbed the media +cell faster than the other two.
- After adding more media, cells were not well located on the scaffold. They being washed away from scaffold.
- After adding more media, some of the scaffold start folating
- contamination

- **RECOMMENDATIONS**
- Mix the gel with cell and print directly in the fume hood. Folating and washing away won't be the problem and the cell will spread evenly in the scaffold.
  - ✓ fully sterilize the 3d printer
  - ✓ UV light will kill the cell

- Contamination – due to unsterile scaffolds and methods. No sterile PBS
- Scaffolds too thick – 8 layer

HA scaffolds – not transparent

We put all the scaffolds under UV light for 1 hr – before checking results

Contamination problem seen after seeding, despite adding antifungal agents,

Unsure if the contamination is fungal or bacterial – 8 layer – HA – discarded it after this stage

Seeding 3h pipette

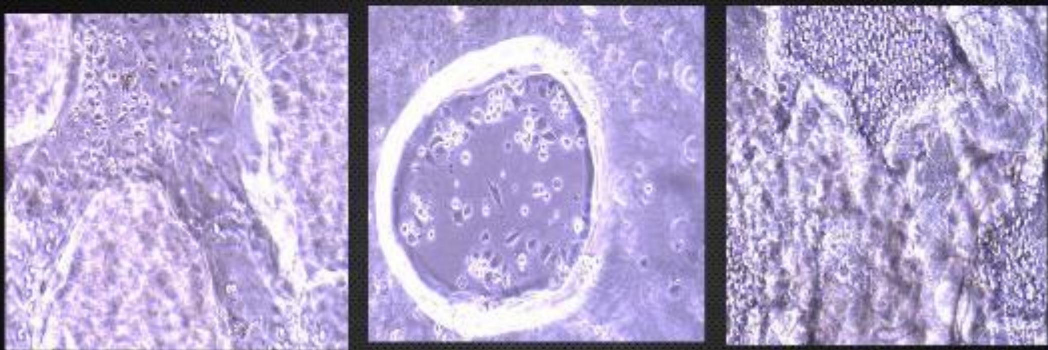
8%GelMa

HA dissolved – Why??

dissolved

8%GelMa+1%Hyaluronic acid(HA)

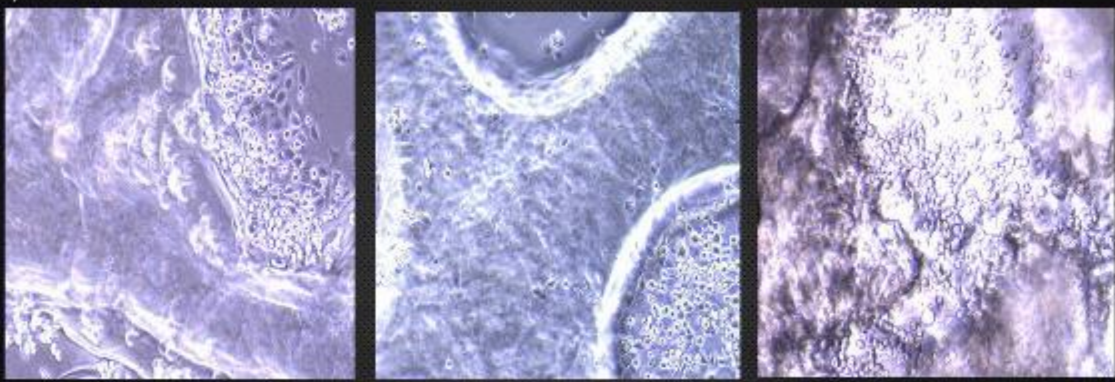
Seeding 3h 2 layer



D 8%GelMa | D 8%GelMa+1%collagen | 8%GelMa+1%Hyaluronic acid(HA)

8%G - cells are on the first layer of scaffold and are spreading into the scaffold not into the pore.
8%G+1%C -cells spreading inside the pore and some cells moving towards scaffold
8%G+1HA – cells are in pore but the scaffold is losing its form

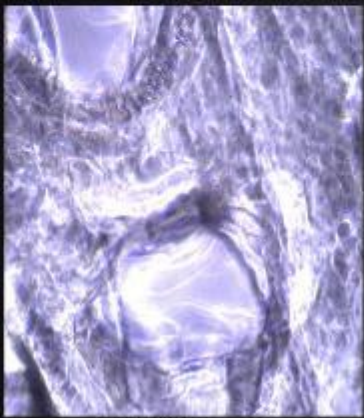
Seeding 3h 4 layer



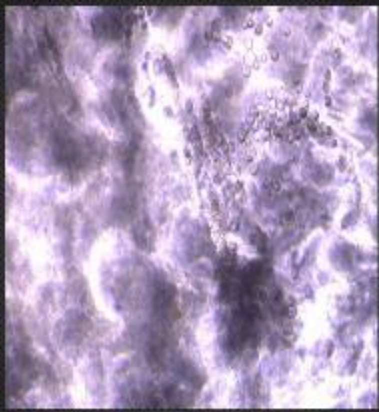
D 8%GelMa | 8%GelMa+1%collagen | 8%GelMa+1%Hyaluronic acid(HA)

8%G – the cells are inside the well plate but are spreading and attaching themselves on the surface of the well plate and scaffold, however the blurred out cells on the first layer of scaffold
8%G+1%C – the cells are mostly found in seeding point, minimal on scaffold after 3 h of seeding
8%G+1% HA – lost its form – cells in the pore

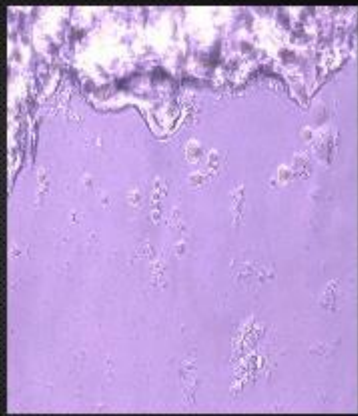
Seeding 3h 8 layer



8%GelMa



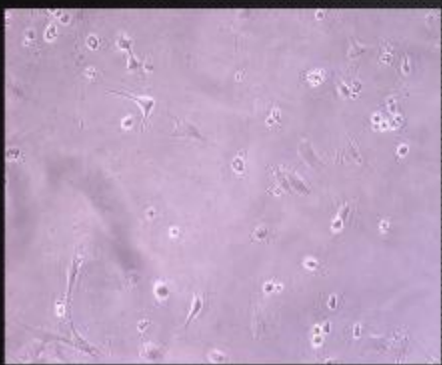
8%GelMa+1%collagen



8%GelMa+1%Hyaluronic acid(HA)

8 layer too thick to look through and the HA scaffold is floating

Seeding 13h pipette



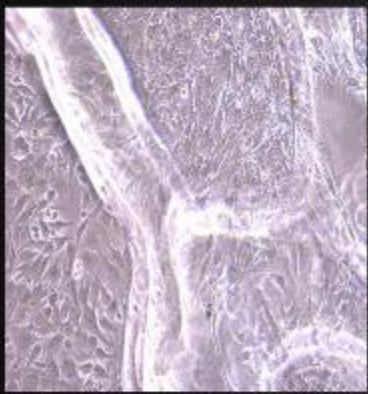
8%GelMa

8%GelMa+1%Hyaluronic acid(HA)

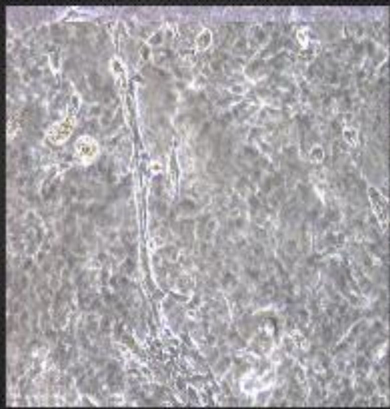
8% G – nothing wrong, healthy cells

8%G+ 1%HA – last time it was dissolved, but some residues were left at the bottom, the cells are growing on those residues, but no cells on plate as no residue of pipette

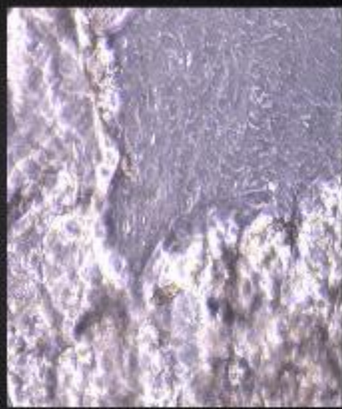
Seeding 13h 2 layer



D 8%GelMa



D 8%GelMa+1%collagen



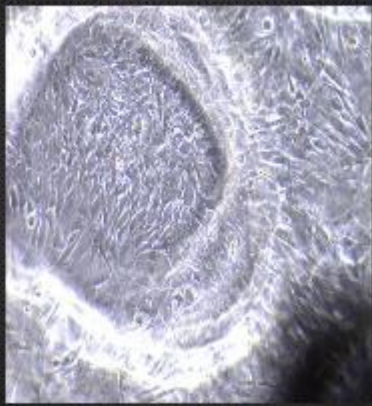
8%GelMa+1%Hyaluronic acid(HA)

Spread more and proliferate more and look healthy
8%G – cells are in pore, but some cells are under the scaffold – this could due to cells making through the scaffold or scaffolds creating trough or floats

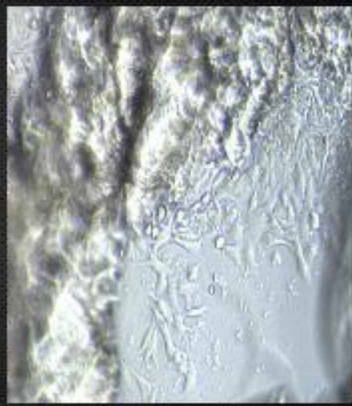
- 8%g+1%C – cell are coming together to form a line, just this but not others – may be its just the scaffold with some curvature?
- 8%G+1%HA – no form of the scaffold but cells are looking healthy

Seeding 13h 4 layer

D 8%GelMa



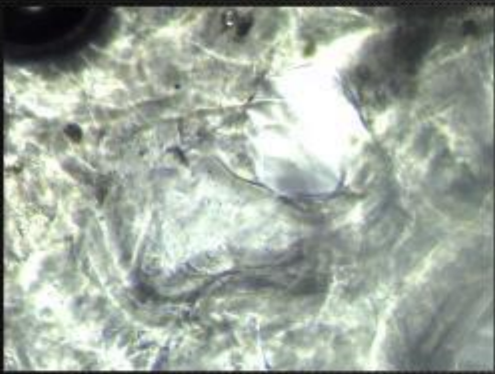
D 8%GelMa+1%collagen



8%GelMa+1%Hyaluronic acid(HA)

- 8%G – similar to 2 layers, looking healthy in the pore.
- 8%G+1%C – cells looking healthy and elongating but more cells on the well plate and pore rather than scaffold.
- 8%G + 1% HA – cells lose structure and cant look at it, so threw it

Seeding 13h 8 layer

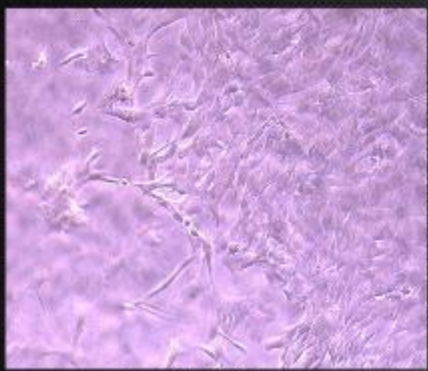


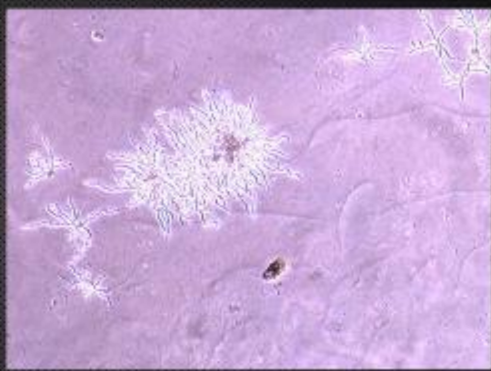
8%GelMa

8%GelMa+1%collagen

8 layer too thick, not visible

Seeding 35h pipette

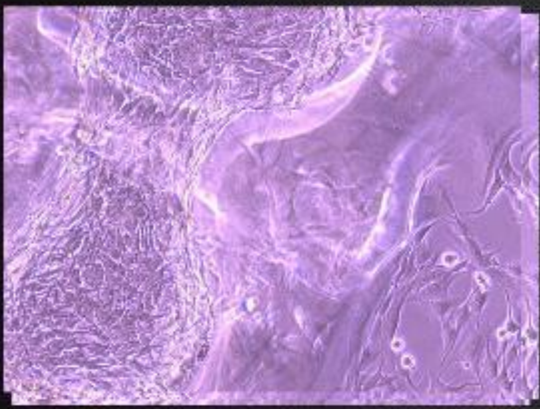




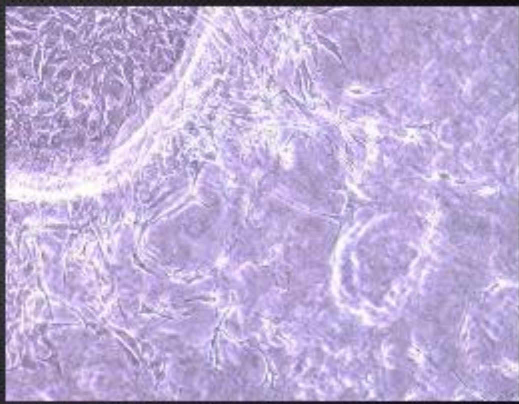
8%GelMa

Cells look fine but just has a bit of fungi observed

Seeding 35h 2 layer



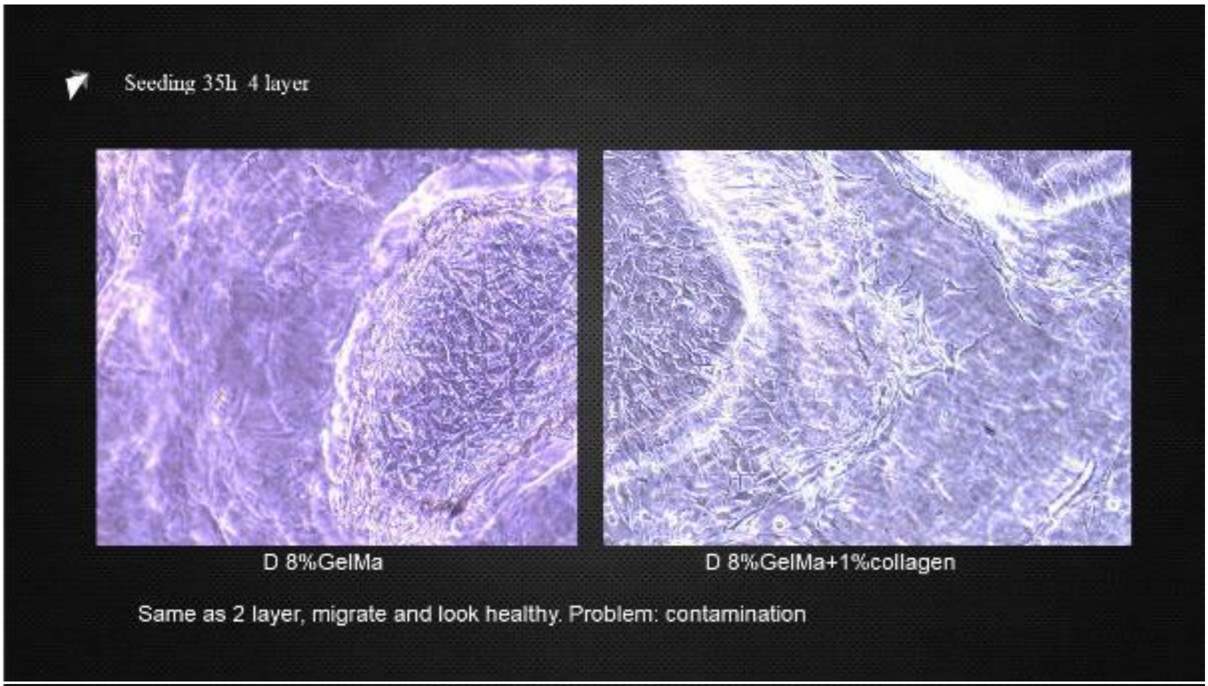
8%GelMa



8%GelMa+1%collagen

8%G - The cells are moving, expand and spread, can grow along the edge of the scaffold – looks like micropatterning (scaffold ends) will help with growing. Dead or unattached cells seen

- 8%G +1%C The cells are growing so they migrate into the scaffold due to space issues, the cells with their cytoplasm first and then drag along nucleus also cells can grow along the edge of the scaffold


Seeding 35h 4 layer
D 8%GelMa
D 8%GelMa+1%collagen
Same as 2 layer, migrate and look healthy. Problem: contamination

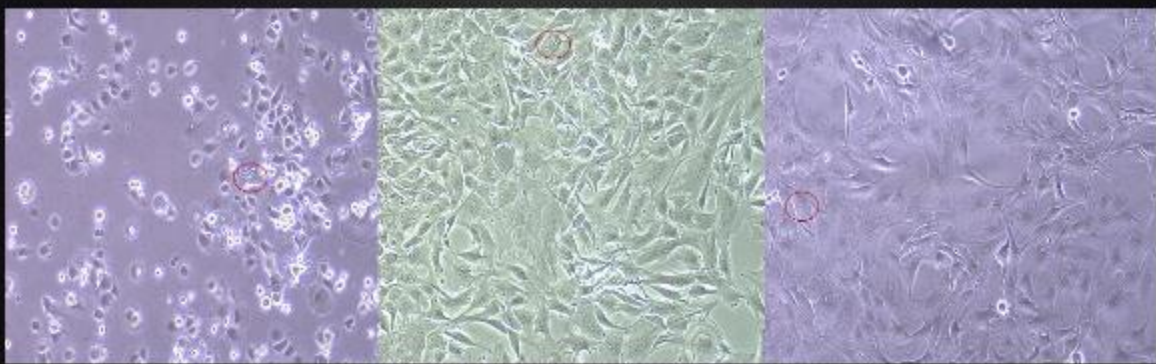

Seeding 35h 8 layer
floating
8%GelMa
8 layer scaffolds – floating and full of contamination

Seeding 35h Problem we met during seeding

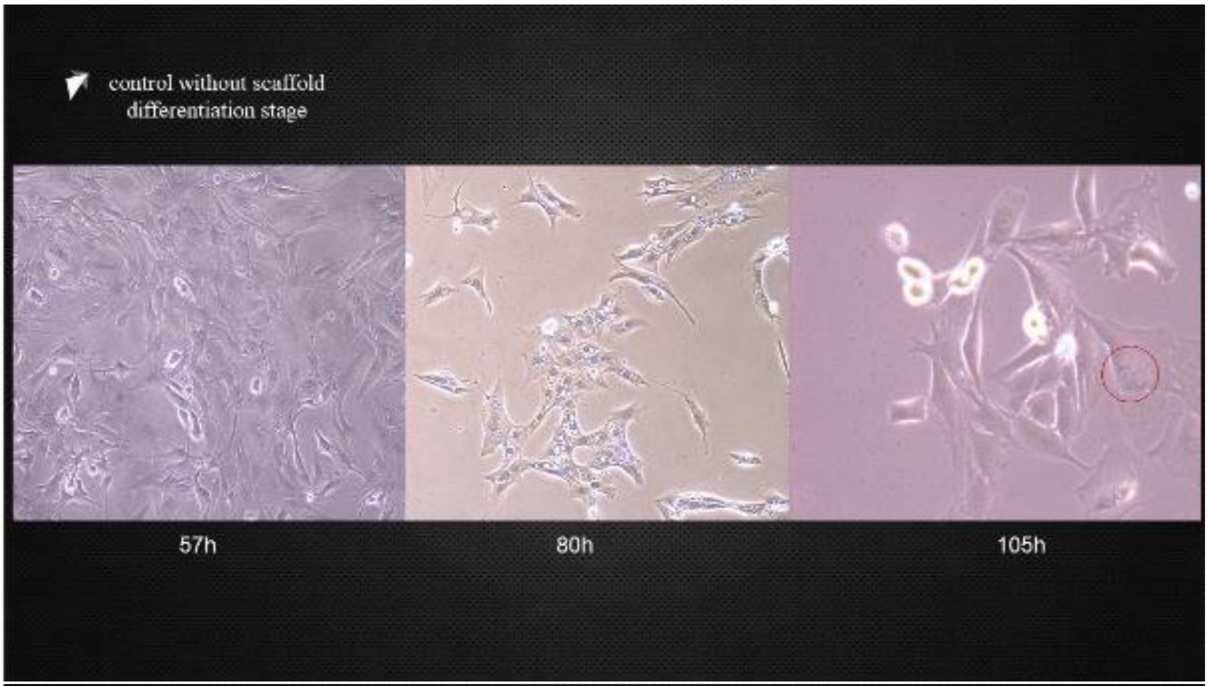
- CONTAMINATION!!!

- After printing store in enthanol
- Make the gel at UC

control without scaffold proliferation stage



3h 13h 35h


control without scaffold
differentiation stage
57h
80h
105h


control without scaffold
differentiation stage
128h
151h
175h

APPENDIX 2

In-vitro meat

Hey, glad to see you around here!

First of all, thank you for taking the time to answer a few questions. You are a great help! 🙂

Please read the information sheet below:

Date Information Sheet Produced:
12 /02/ 2019

Project Title
Perception of in-vitro meat (IVM) by New Zealand consumers.

An Invitation:

Dear Participants,

My name is Maya Murthy Malavalli, a student of Master of Science. I am conducting a research project on in-vitro meat (IVM) in the Faculty of Health and Environmental Science at the Auckland University of Technology. I would like to invite you to participate in a study that seeks to understand the perception of in-vitro meat (IVM) among New Zealand consumers.

Participation in this research is completely voluntary, anonymous, and confidential. You are under no obligation to complete the questionnaire, and you have the freedom to withdraw at any stage without question.

Please note that the participants will be made to go through infographics with some images and content which may be disturbing.

This Participation Information Sheet will help you decide if you would like to participate in this study. It explains why we are doing this study, how you were chosen for this invitation, how your privacy is protected and what happens after the study is completed. You do not have to decide today whether or not you will participate in this study. Please feel free to discuss your decision with family or friends.

What is the purpose of this research?
The aim of this project is to understand the perception of in-vitro meat by New Zealand consumers.

Am I eligible to participate in this research?
We welcome all individuals to participate in this study that meets the following criteria:
-You are over the age of 18
-You have lived here for more than 3-5 years, preferably but not mandatory.
-You consume meat more than 3 times in a week (OR)
-You are a non-vegetarian who is looking at sustainable meat alternatives (OR)
-You are a vegetarian who is looking for better protein intake.

How do I agree to participate in this research?
Your participation in this research is voluntary (it is your choice), and whether or not you choose to participate will neither advantage nor disadvantage you. You can withdraw from the study at any time.

What will happen in this research?
If you take part in this project, you will be asked to answer an online questionnaire on in-vitro meat, to understand its perception and willingness to try by New Zealand consumers.

What are the discomforts and risks?
It is highly unlikely that you will feel discomfort or are faced with any risk. It is not anticipated that you will experience discomfort or risk in this study.

What are the benefits?
This research will give more insight and understanding of the perception of in-vitro meat among New Zealand consumers.

How will my privacy be protected?
Your responses are anonymous and confidential. Upon completion of the questionnaire, the data will be combined with all the other participants' data. Your anonymity is completely assured throughout the entire study.
Please note that your responses to the questionnaire will not be stored indefinitely. The data will be destroyed upon completion of data analysis.

What are the costs of participating in this research?
There will be no cost in this study if you wish to take part in. We understand that time is an important factor and we value your participation and responses.

What opportunity do I have to consider this invitation?
Your participation in this study is completely voluntary. If you require further information or want to ask questions about this research, please contact me via email. If you would like to discuss the requirements please, email me indicating a suitable time and I will reply by email in a reasonable time frame.

Will I receive feedback on the results of this research?
General feedback will be given to the participants at the end of the experimental session. Participants can email the researcher to receive a summary of the findings if they are interested.

What do I do if I have concerns about this research?
Please contact Project Supervisor: Nazimah Hamid (nazimah.hamid@aut.ac.nz).
Concerns regarding the conduct of the research should be notified to the Executive Manager of AUTEC, Kate O'Connor, Room WU407, Level 4, WU Building, 46 Wakefield Street, Auckland 1010, Private Bag 92006, Auckland 1142, Internal Mail Code D-88, Phone +64 9 921 9999 extn: 6038, Email: ethics@aut.ac.nz.

Whom do I contact for further information about this research?
Please keep this Information Sheet. You may contact the research team members using the details shown below:

Researcher Contact Details:
Maya Murthy Malavalli
Maya.mayamurthy@gmail.com **(OR)**
zxj3359@autuni.ac.nz
0224783885

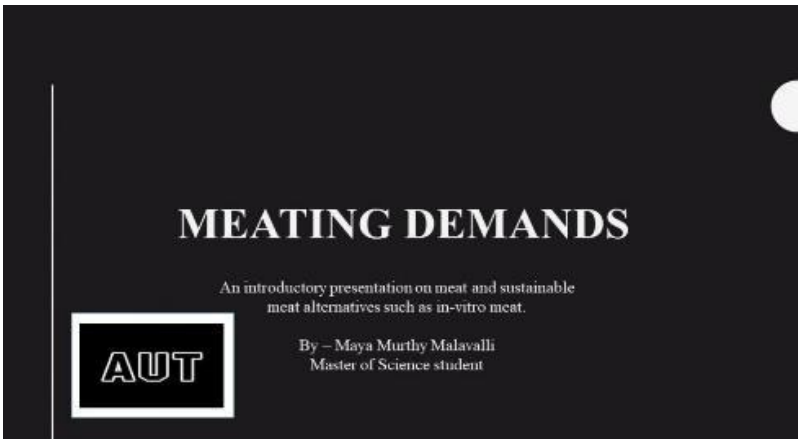
Project Supervisor Contact Details:
Nazimah Hamid
nazimah.hamid@aut.ac.nz
+64 9 921 9999 ext 6453

Approved by the Auckland University of Technology Ethics Committee on 28 Mar 19, AUTEC Reference number 19/68
Completion of the attached questionnaire will be taken as indicating your consent to participate.

NOTE:
Please note that you may use the horizontal scrollbar while going through the powerpoint slides. Always use accelerometer on your phone to change the orientation of the screen (landscape mode)
Please select the continue or next arrow button " -->" to continue and maximize your screen for best view.
Always click the next button at the bottom of each page after answering the question(s) on that page.
Click "Submit" at the end of the questionnaire.
Please DO NOT click the "X" button until you have submitted the survey or we may not receive your responses correctly!

Thank you very much for your participation and time. 🙂

Q1.2

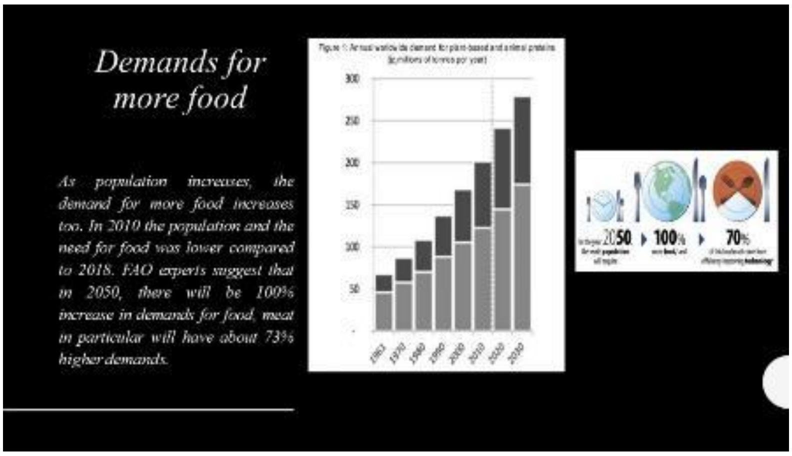


Q1.3

Food can be anything…

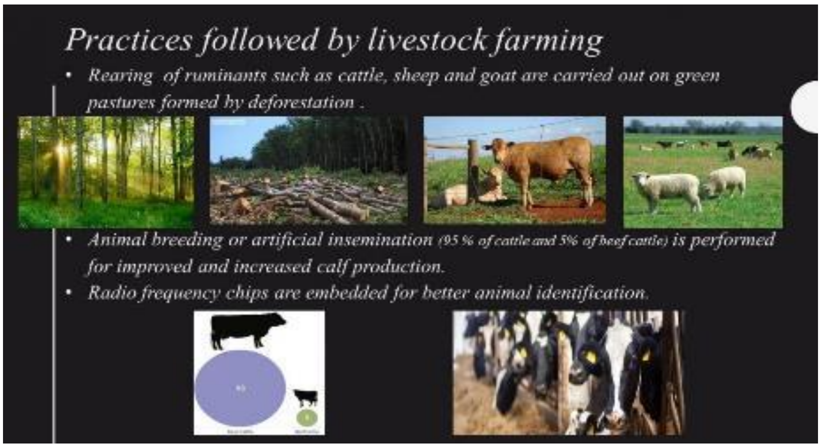
Q1.4

Agriculture
Aquaculture
Food is obtained by the following methods
Livestock farming

Q1.5

Demands for more food
As population increases, the demand for more food increases too. In 2010 the population and the need for food was lower compared to 2018. FAO experts suggest that in 2050, there will be 100% increase in demands for food, meat in particular will have about 73% higher demands.


Livestock farming
• Domesticated animals such as cattle beef, pigs, sheep, goat, duck and chicken for meat consumption.
• Estimated demands by 2050 is 70% more than 2018
cattle
horse
goat

Q1.6


Practices followed by livestock farming
• Rearing of ruminants such as cattle, sheep and goat are carried out on green pastures formed by deforestation .
• Animal breeding or artificial insemination (95 % of cattle and 5% of beef cattle) is performed for improved and increased calf production.
• Radio frequency chips are embedded for better animal identification.

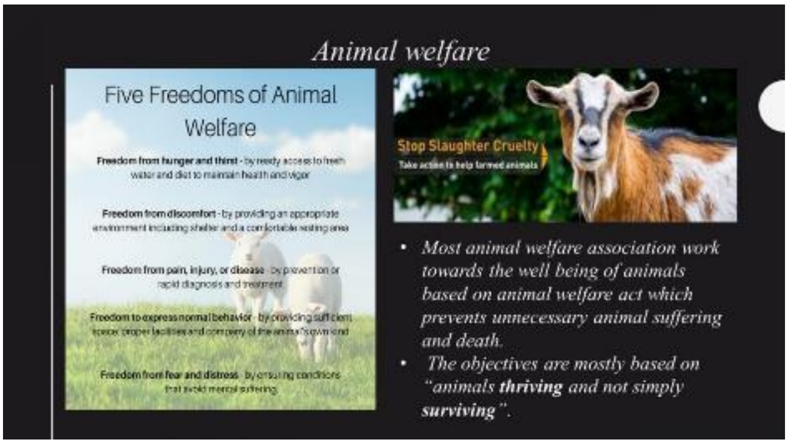
Q1.7


Practices followed by traditional meat industry
Following from the previous methods, farm animals are handed over to traditional meat industry or slaughter houses. These animals are further
• Caged

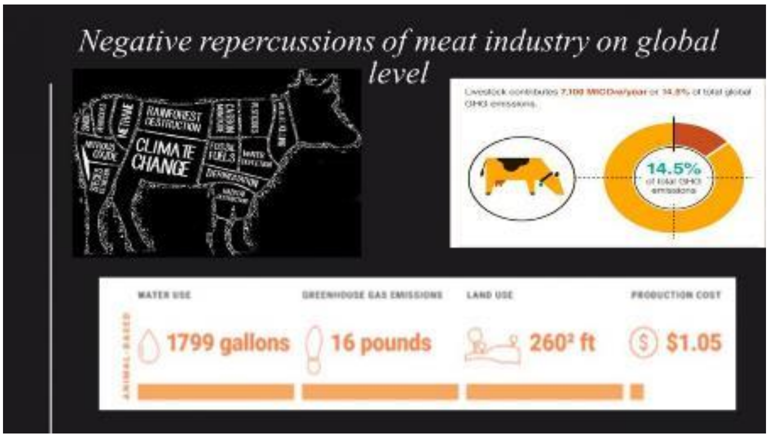
Q1.8

Q1.9

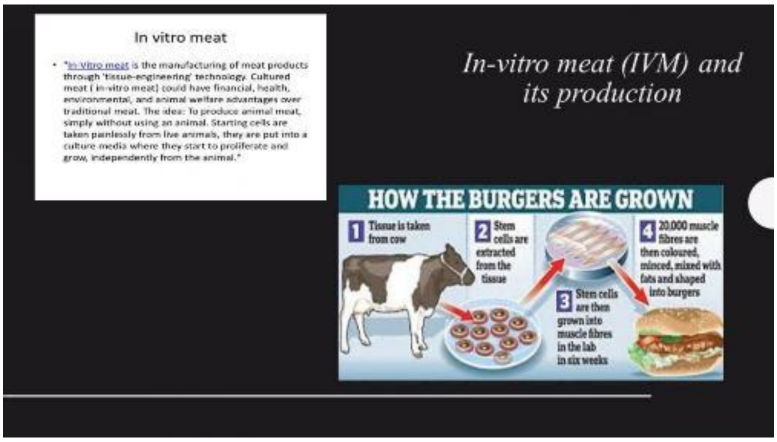
Q1.10



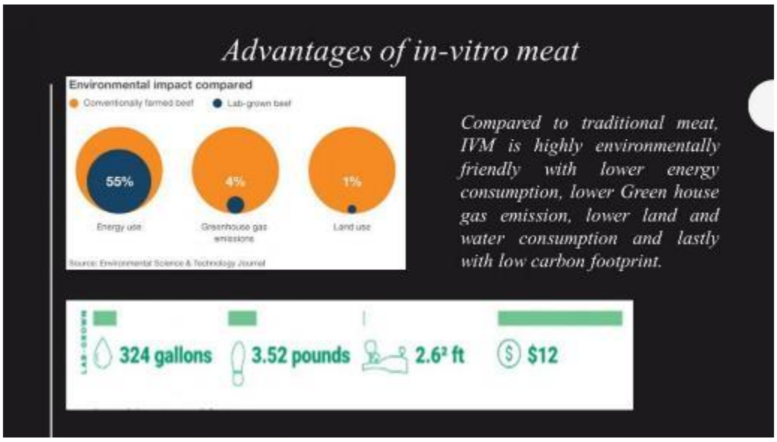
Q1.11



Q1.12



Q1.13



Q1.14

Q1.15

Q1.16

Q2.1

Please choose the closest age group you belong to.

- 18 - 25
- 26 - 35
- 36 - 45
- 46 - 55
- 56 - 65
- 65 and above

Q2.2

What is your gender?

- Male
- Female
- Prefer not to say

Q2.2

What is your gender?

- Male
- Female
- Prefer not to say

Q2.3

Please indicate your highest qualification?

- High School
- Diploma or certification
- Bachelor degree
- Master degree
- Postgraduate degree

Q2.4

What is your occupation?

- Trade
- Engineer
- Banking
- Administration
- Healthcare professional
- Science technician
- Student
- Prefer not to say

Q2.5

What is your household income?

- < 20,000$ per year
- approx. 50,000$ per year
- 50,000$ - 70,000$ per year
- 70,000$ - 90,000$ per year
- 90,000$ - 120,000$ per year
- >120,000$ per year
- Prefer not to say

Q2.6

Are you working...

- Full Time
- Part Time
- Retired
- Unemployed

Q2.7

What is your current Marital status?

- Single
- Married
- Separated
- Divorced
- De facto
- In a civil union
- Prefer not to say

Q2.8

What is your household size?

- One person
- Two-four people
- Four-eight people
- More than 8 people
- Prefer not to say

Q2.9

What is your ethnicity?

- Maori
- European
- Chinese
- Indian
- Pacific islander
- Fiji Indian
- Other
- Prefer not to say

Q2.10

Please indicate your nationality

Q2.11

Are you a religious person?

- Yes
- No
- Prefer not to say

Q2.12

Please mention your email address. Your email address will be used for rewards purpose only, it will be confidential and your privacy will not be breached at any times.

Q3.1

Which of the following describes your eating **habits**?

- Vegetarian
- Non-vegetarian
- Flexitarian - a person who has a primarily vegetarian diet but occasionally eats meat or fish.
- Pescatarian - a person who does not eat meat but does eat fish.
- Others

Q3.2

How **often** do you eat meat?

- Never
- Rarely, occasional meat eater
- Sometimes
- Most of the time
- Always

Q3.3

Which **type of meat** do you prefer the most? Check all that applies

- Poultry (chicken)
- Pork
- Beef
- Fish and other seafood
- Others

Q3.4

Who is responsible for doing the **grocery** shopping within your household?

(Grocery- Any household item or items of food)

- Myself
- Partner
- Parents
- Shared responsibility
- Others

Q3.5

Why do you prefer meat? Check all that applies.

- Flavour and appearance
- Nutrition profile
- Price
- Fashion
- Others

Q3.6

Do you think you consume **meat** just **for its taste, flavour and appearance**?

- Strongly agree
- Somewhat agree
- Neither agree nor disagree
- Somewhat disagree
- Strongly disagree

Q3.7

Do you think you would consume meat for **protein intake**?

- Strongly agree
- Somewhat agree
- Neither agree nor disagree
- Somewhat disagree
- Strongly disagree

Q3.8

What are the **aspects** you consider when purchasing meat? Select all that applies.

- Taste
- Texture
- Nutrition
- Price
- Sale
- Freshness
- Country of origin (meat)
- Packaging
- Advertisement
- Expiry date

Q3.9

How important is **health star** rating to you when buying processed meat?

- Extremely important
- Very important
- Moderately important
- Slightly important
- Not at all important

Q3.10

Who is the **purchaser** of meat in your family?

- Myself
- Family members such as grandmother, mother, aunt, wife or sister
- Friend / flatmate
- Partner
- Others

Q3.11

What does your **meat intake** look like?

- Once a month
- Once in a fortnight
- Once a week
- Every meal
- Never

Q3.12

When do you usually eat meat? When I am ....

- Hungry
- Bored
- Crave
- Sad
- Happy

Q3.13

Do you feel that your **emotions** are **uplifted** every time you eat meat?

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly agree

Q3.14

Do you think you have an **emotional eating disorder**?

(Emotional eating is eating to satisfy emotional needs, rather than to satisfy physical hunger.)

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly agree

Q3.15

Do you eat meat when you are **emotionally upset**?

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly agree

Q3.16

Which of the following would you binge eat when you suffer from **emotional eating**? Select one or all that applies

- Food such as fish and chips, Fried chicken and chips, KFC Hot rods, Beef Baconator, Angus beef burger, Big Mac, Gourmet beef burger
- Restaurant bought stir fry or curries with meat
- Homemade stir fry or curries with meat
- Sugary and carbs filled snacks
- Chocolates

Q3.17

Have you ever thought of **giving up meat**?

- Never
- Rarely
- Sometimes
- Often
- Always

Q3.18

Do you think you have had any **meatless days**?

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly agree

Q3.19

What are the reasons for your **meatless days**?

- Dietary changes
- Religious reasons
- Unavailability of meat
- Conscious effort to quit meat due to its negative side effects and animal welfare reasons
- Others

Q4.1

How important is the **environment** to you?

- Not at all important
- Slightly important
- Moderately important
- Very important
- Extremely important

Q4.2

Are you aware of the **negative environmental** effects of the conventional meat industry?

(Conventional meat industry - meat industry which produces meat such as beef, poultry, lamb and fish by traditional methods such as animal rearing and slaughtering.)

- Not at all aware
- Somewhat aware
- Moderately aware
- Very aware
- Extremely aware

Q4.3

Do you think that the traditional meat industry contributes to global issues such as **greenhouse gas emission** and **changes in climate**?

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly agree

Q4.4

Are you aware of the fact that the **conventional meat industry has a higher carbon footprint** compared to other meat alternatives?

(Carbon footprint -the amount of carbon dioxide released into the atmosphere as a result of the activities of a particular individual, organization, or community.
Conventional meat industry - meat produced by rearing and slaughtering farm animals.)

- Not at all aware
- Slightly aware
- Moderately aware
- Very aware
- Extremely aware

Q4.5

Are you aware of the terms such as **sustainability** and **sustainable environment**?

(Sustainability - the ability to be maintained at a certain rate or level, to avoid the depletion of natural resources to maintain an ecological balance
Environmental sustainability - A state in which the demands placed on the environment can be met without reducing its capacity to allow all people to live well, now and in the future.)

- Not at all aware
- Slightly aware
- Moderately aware
- Very aware
- Extremely aware

Q4.6

Are you aware of sustainable **meat alternatives**?

- Not at all aware
- Slightly aware
- Moderately aware
- Very aware
- Extremely aware

Q4.7

Do you think you are **open to technologies** related to the food industry?

- Strongly disagree
- Disagree
- Neither agree nor disgree
- Agree
- Strongly agree

Q4.8

Are you familiar with **meat analogs** such as plant-based protein, insect protein, In vitro meat, vegan fish and fishless seafood?

(Mycoprotein – protein derived from fungi especially produced for human consumption).

- Not at all familiar
- Slightly familiar
- Moderately familiar
- Very familiar
- Extremely familiar

Q4.9

Do you believe in **animal welfare**?

(Animal welfare means how an animal is coping with the conditions in which it lives. An animal is in a good state of welfare if (as indicated by scientific evidence) it is healthy, comfortable, well nourished, safe, able to express innate behavior, and if it is not suffering from unpleasant states such as pain, fear, and distress.)

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly agree

Q4.10

Are you **familiar** with technologies such as Cellular agriculture / In-vitro meat (IVM) technology and tissue engineering ?

- Not at all familar
- Slightly familiar
- Moderately familiar
- Very familiar
- Extremely familiar

Q4.11

"In-vitro meat (**IVM**) is also known to have the **least carbon footprint** compared to traditional beef industry." Do you think IVM is the most suitable sustainable meat alternative?

(Carbon footprint -the amount of carbon dioxide released into the atmosphere as a result of the activities of a particular individual, organization, or community.)

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly agree

Q4.12

"In-vitro meat (**IVM**) is known to have **no adverse effects on the environment**, unlike conventional meat industry." Do you think IVM is a better and environmentally friendly option?

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly agree

Q4.13

"Slaughterhouses use stunning and percussion techniques which contribute to animal suffrage, whereas in-vitro meat (IVM) is cruelty-free". Do you agree that IVM is **animal-friendly**?

(Stunning is the process of rendering animals immobile or unconscious, with or without killing the animal, when or immediately prior to slaughtering them for food).

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly agree

Q5.1

Do you think you would likely consume in-vitro meat (IVM) if your **religious beliefs permitted**?

- Definitely yes
- Probably yes
- Might or might not
- Probably not
- Definitely not

Q5.2

Do you think you would eat in-vitro meat (IVM) if the **religious leaders** informed you?

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly agree

Q5.3

In some religions, the intellectual level of the animal to be slaughtered for meat purposes are considered, such as "only those animals with lower intellectual capacity and pain sensation are to be slaughtered" whereas, **in-vitro meat (IVM) does not require any animal slaughter at all**.

Do you think in-vitro meat (IVM) is a better meat alternative than conventional meat?

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly agree

Q5.4

Do you think you would opt for in-vitro meat (IVM) if there will be **halal or kosher** options available?

[Halal - refers to what is permissible or lawful in traditional Islamic law
Kosher - Kosher foods are those that conform to the Jewish dietary regulations of kashrut (dietary law)].

- Strongly disagree
- Disagree
- Neither disgree nor agree
- Agree
- Strongly agree

Q6.1

Do you think you will still buy conventional meat although it has a **higher carbon footprint**?

(Conventional meat - meat such as beef, poultry, lamb and fish obtained by traditional methods such as animal rearing and slaughtering).

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly agree

Q6.2

Do you think you would give up meat **for** the sake of **animals**?

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly Agree

Q6.3

Do you think you would buy plant-based products if it had a **better health star rating** compared to meat products?

- Definitely yes
- Probably yes
- Might or might not
- Probably not
- Definitely not

Q6.4

Do you think would you consume in-vitro meat (IVM) if it is produced by **big multinational companies without any hormones & antibiotics**?

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly Agree

Q6.5

Do you think would you consume in-vitro meat (IVM) if it was **produced by small industries using cells obtained directly from farm animals**?

- Definitely yes
- Probably yes
- Might or might not
- Probably not
- Definitely not

Q6.6

Do you think would you consume in-vitro meat (IVM) if it was locally made on a **weekly basis**?

- Strongly disagree
- Disgree
- Neither agree nor disagree
- Agree
- Strongly agree

Q6.7

Do you think you would switch over to in-vitro meat (IVM) if your **family** switched over to in-vitro meat / clean meat?

- Definitely yes
- Probably yes
- Might or might not
- Probably not
- Definitely not

Q6.8

Do you think you would buy in-vitro meat (IVM) if **farm animals were extinct** and IVM was the only source of meat?

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly Agree

Q6.9

Do you think you would try in-vitro meat (IVM) for its **nutritional profile**, do you think it is an ideal meat alternative?

(The nutritional profile of IVM is high in protein, low fat, low cholesterol and fiber).

- Strongly disagree
- Disagree.
- Neither agree nor disagree
- Agree
- Strongly agree as it sounds healthy

Q6.10

Do you think you will buy in-vitro meat (IVM) over traditional meat even though it **tastes inferior**?

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly agree

Q6.11

Do you think you would buy in-vitro meat (IVM) over conventional meat **if it appeared and tasted better**?

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly agree

Q6.12

Do you think you would buy in-vitro meat (IVM) over traditional meat if the **flavour was enhanced using food additives**?

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Agree

Q6.13

If IVM was to be improved and had matched the traditional **meat sensory qualities**, would you purchase it?

(Sensory qualities such as taste, flavour, smell, appearance and texture)

- Definitely yes
- Probably yes
- Might or might not
- Probably not
- Definitely not

Q6.14

Do you think you would consume in-vitro meat (IVM) over traditional meat if it made you **feel good**?

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly agree

Q6.15

How would you want in-vitro meat (IVM) to be if it was sold as a **commercial product**?

- Processed food
- Ready to eat food
- Long shelf life food
- Fresh food

Q6.16

Most of the western and European countries are switching to in-vitro meat (IVM), nevertheless, IVM is still in early stages in New Zealand. Do you think you would switch to IVM if **New Zealanders switched over to IVM to be on par with other Western and European countries**?

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly agree

Q6.17

Do you think you would try in-vitro meat (IVM) if it was **legalized by the NZ government**? If there were rules governing the production and marketing of IVM?

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly agree

Q6.18

In-vitro meat (IVM) has a lot of **stigmas** associated with it as it is a new product, do you think you will try it over conventional meat?

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly agree

Q7.1

Do you think you were **aware** of the concept of in-vitro meat (**IVM**) prior to this study?

- Not at all aware
- Slightly aware
- Moderately aware
- Very aware
- Extremely aware

Q7.2

Do you think in-vitro meat (IVM) is **artificial**?

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly agree

Q7.3

Do you think the idea of in-vitro meat (IVM) is **dangerous**?

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly agree

Q7.4

Do you associate the idea of in-vitro meat (IVM) with something **negative** (e.g. lab-grown, fake and GMO)?

(GMO - geneitcally modified organism)

- Definitely yes
- Probably yes
- Might or might not
- Probably not
- Definitely not

Q7.5

Do you think in-vitro meat (IVM) is good for **animal welfare**?

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly agree

Q7.6

Do you think in-vitro meat (IVM) will lead to **farm animal extinction**?

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly agree

Q7.7

Do you think in-vitro meat (IVM) will become **more popular than conventional meat**?

- Strongly agree
- Agree
- Neither agree nor disagree
- Disagree
- Strongly disagree

Q7.8

Do you think promoting in-vitro meat (IVM) will lead to **cannibalism**?

Human cannibalism is the act or practice of humans eating the flesh or internal organs of other human beings

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly agree

Q7.9

Do you think in-vitro meat (IVM) is **unnatural**?

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly agree

Q7.10

Do you think in-vitro meat (IVM) will **solve environmental and food security issues**?

- Strongly disagree
- Agree
- Neither agree nor disagree
- Agree
- Strongly agree

Q7.11

Do you think in-vitro meat (IVM) is a suitable **sustainable meat alternative**?

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly Agree

Q7.12

Do you think in-vitro meat (IVM) could have **inferior taste/texture** than conventional meat as it does not have fat cells?

(Fat cells contribute to taste profile such as juicy and meaty)

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly agree

Q7.13

Do you think in-vitro meat (IVM) is **inferior meat** as it is man-made and lacks dietary fiber?

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly agree

Q7.14

Do you think that in-vitro meat (IVM) is **natural**?

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly agree

Q7.15

Do you think **in-vitro meat (IVM)** will offer good culinary experiment as it is **neutral in taste**, so you can add desired taste /flavor?

- Definitely yes
- Probably yes
- Might or might not
- Probably not
- Definitely not

Q7.16

Do you think **in-vitro meat (IVM)** is a **waste of resources**?

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly agree

Q7.17

Do you think in-vitro meat (IVM) technology is **unsafe**?

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly agree

Q7.18

If in-vitro meat (IVM) became popular in the future and everyone switched to IVM, do you think people will stop eating conventional meat which may result in **increased livestock population globally**?

(Livestock population - the number of cattle and sheep population)

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly agree

Q7.19

If in-vitro meat (IVM) became popular, do you think the overall **meat consumption rate (both IVM and conventional meat) would increase** with no significant impacts on the environment and animal cruelty?

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly agree

Q7.20

Do you think you will buy in-vitro meat (IVM) over conventional meat as it is almost organic, **cruelty-free and sustainable meat**?

- Definitely yes
- Probably yes
- Might or might not
- Probably not
- Definitely not

Q8.1

Do you think you would buy in-vitro meat (**IVM**) **for** your **family** if it was **commercially available**?

- Definitely yes
- Probably yes
- Might or might not
- Probably not
- Definitely not

Q8.2

Do you think you would buy **in-vitro meat (IVM) over conventional meat** if it was commercially available?

- Definitely yes
- Probably yes
- Might or might not
- Probably not
- Definitely not

Q8.3

Do you think you would buy in-vitro meat (IVM) if it is **affordable**?

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly agree

Q8.4

Do you think you would buy in-vitro meat (IVM) if it would be **cheaper** than conventional meat? For example, if IVM was **about 20$ per kg**.

(Reports suggest that it costs about 71.75$ NZD for an IVM burger patty)

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly agree

Q8.5

Do you think you would buy in-vitro meat (IVM) over conventional meat even though it is **expensive**?

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly agree

Q8.6

Do you think you would buy in-vitro meat (IVM) **regularly**?

- Probably yes
- Definitely yes
- Might or might not
- Probably not
- Definitely not

Q9.1

In-vitro meat (IVM) is called by many names, which of the following names sounds **pleasant and more appealing**? Select one or more than one option

- In-vitro meat
- Lab meat
- Synthetic meat
- Artificial meat
- Clean meat

Q9.2

Are you familiar with **plant-based meat** brands such as Impossible burger, Meatless chicken, Beyond meat, Eggless egg?

- Not at all familiar
- Slightly familiar
- Moderately familiar
- Very familiar
- Extremely familiar

Q9.3

Do you think you would be more open to in-vitro meat (IVM) if **social media promoted it as the best sustainable meat alternative**?

- Definitely yes
- Probably yes
- Might or might not
- Probably not
- Definitely not

Q9.4

If the media informed the public about the **negative repercussions of the traditional meat industry and the global advantages of in-vitro meat (IVM) technology**, do you think your opinion would change? Would you be more familiar about IVM?

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly agree

Q9.5

Do you think you would **accept** in-vitro meat (**IVM**) **samples if it was offered at the supermarket** with a popular product or with other sustainable alternatives such as plant-based egg and plant-based milk?

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly agree

Q9.6

Do you think you would buy in-vitro meat (IVM) if it is labeled as **guilt-free** meat?

- Definitely yes
- Probably yes
- Might or might not
- Probably not
- Definitely not

Q9.7

Do you think you would buy/consume in-vitro meat (IVM) if it was **regulated** by government agencies such as FDA, FAO, MPI, FSANZ and MBIE?

(FDA - Food and Drug Administration, US.
FAO - Food and Agriculture Organization, United Nations.
MPI - Ministry of Primary Industries, NZ.
FSANZ - Food Standards Australia and New Zealand.
MBIE - Ministry of Business, Innovation and Employment, NZ)

- Definitely yes
- Probably yes
- Might or might not
- Probably not
- Definitely not

Q10.1

Do you think in-vitro meat (IVM) has any **health and safety concerns**?

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly agree

Q10.2

Do you think **in-vitro meat (IVM)** is likely to cause any **disease**?

- Not at all likely
- Somewhat likely
- Moderately likely
- Very likely
- Extremely likely

Q10.3

Do you think in-vitro meat (IVM) is **cancerous** as it involves stem cells?

(Stem cells - an undifferentiated cell of a multicellular organism which is capable of giving rise to indefinitely more cells of the same type, and from which certain other kinds of cell arise by differentiation).
OR
(Stem cells - cells used in tissue engineering).

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly agree

Q10.4

Do you think in-vitro meat (IVM) will have any **food safety risk**?

(Food safety risk - the ability of a food sample to cause illness or food poisoning).

- Definitely yes
- Probably yes
- Might or might not
- Probably not
- Definitely not

Q11.1

Do you think you would consider in-vitro meat (IVM) as an alternative if it does not involve **tissue engineering**?

(Tissue engineering - Tissue engineering is the use of a combination of cells, engineering and materials methods, and suitable biochemical and physiochemical factors to improve or replace biological tissues. Tissue engineering involves the use of a tissue scaffold for the formation of new viable tissue for a medical purpose).

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly agree

Q11.2

Do you think you would consider in-vitro meat (IVM) as an alternative if it will be **produced on an everyday basis**?

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly agree

Q11.3

Do you think you would consider IVM as an alternative **if it did not involve stem cells**?

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly agree

Q11.4

Do you think in-vitro meat (IVM) should be **produced by government-approved agencies**?

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly agree

Q11.5

Do you think in-vitro meat (IVM) should be **produced in NZ**? As it is produced by developed countries such as the United States and other Europe countries.

- Definitely yes
- Probably yes
- Might or might not
- Probably not
- Definitely not

Q11.6

Do you think you would consider buying in-vitro meat (IVM) produced by **smaller start-up companies**?

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly agree

Q11.7

Do you think in-vitro meat (IVM) is **ethical?**

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly agree

Q11.8

Would you be more open to in-vitro meat (IVM) if the production companies made strict self-**regulatory institutional policies** for sustainability, marketing, production and food safety policies?

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly agree

Q11.9

Would be more open to in-vitro meat (IVM) if academicians and researchers came up with a **natural way of production**?

- Strongly disagree
- Disagree
- Neither agree nor disagree
- Agree
- Strongly agree

Q11.10

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