Please cite the Published Version

Wilson, Dana, Tsigkou, Olga, Bosworth, Lucy and Jones, Celina (2023) Leather-like materials by cellular agriculture. Textile Progress, 55 (1). pp. 1-45. ISSN 0040-5167

DOI: https://doi.org/10.1080/00405167.2023.2188835

Publisher: Taylor & Francis

Version: Accepted Version

Final Word Count: 20421

Production of Leather-like Materials by Cellular Agriculture

Authors/Affiliations

Dana Wilson[a], Olga Tsigkou[a], Lucy Bosworth[b], Celina Jones[a]

[a] Department of Materials, School of Natural Sciences, The University of Manchester, Oxford Road, Manchester, M13 9PL, UK

[b] Department of Eye and Vision Science, Institute of Life Course and Medical Sciences, The University of Liverpool, 6 West Derby Street, Liverpool, L7 8TX, UK

Abstract

Leather, a popular material in a wide array of industries, is traditionally sourced from animal hides. The scale of production has increased over time, leading to ever-greater concerns about the environmental, ethical and health impacts of leather manufacture. The substantial resources required, plus the pollution and waste generated, pose serious doubts over the sustainability of existing production systems and their ability to meet the increasing demand for leather-like materials. To address these issues, alter- natives to leather have been developed. Up to now though, these materials have been unable to perform as well as genuine leather, either mechanically, aesthetically or texturally. Some of the polymer-based alternatives may even be more harmful to the environment than leather itself. The need for a more-suitable leather substitute has coincided with the emergence of cellular agriculture technologies. In the future, it is hoped that leather-like materials may be engineered from collagen created by cellular agriculture, instead of relying upon animal slaughter. Such a material could offer great design, sustainability, environmental and ethical benefits over real leather. Whilst there is significant potential, more investment in research and development is needed before the technology can be considered sufficiently well developed. So far, tissue engineering techniques applied from clinical fields have proven too costly and inefficient for scaling up, but work has already commenced to identify sources of collagen and cell growth media that are less animal-dependent and not so expensive.Even so, more-efficient methods of controlling the collagen network structure still need to be created. The new round of research is therefore expected to focus upon increasing cell-culture efficiency using, for example, specialised bioreactors.

KEYWORDS

Leather; leather-like; biomaterial; sustainability; tissue engineering; collagen; cellular agriculture

1. Introduction

Leather is classically defined as a material produced upon the preservation of animal hides (Sharphouse, 1995a). The material is used in a wide variety of industries, including clothing, footwear, furnishing and automotive. The high global demand for leather is due to its unique properties, such as durability, lustrous texture and breathability (Covington, 2011a). The scale of production of leather has increased over time, leading to concerns about negative environmental, ethical and health impacts of various aspects of leather manufacture. For example, the resource demands, the pollution and the waste generated, pose serious doubts over the sustainability of existing production systems and their capacity to meet ever-increasing demand. Production of leather poses ethical issues surrounding animal welfare before slaughter and environmental concerns throughout the material's life cycle (Tasca and Puccini, 2019). Consumers are increasingly conscious of such consequences surrounding their product choices (Thomas, 2019). There is more significant pressure on, plus competition between, manufacturers to provide consumers with the sustainable, ethical and high-quality products they desire.

To address such issues, leather substitutes have been developed, deriving from natural and synthetic polymers. The most widely used plastic alternatives are, however, heavily reliant upon unsustainable fossil fuels and cannot biodegrade as readily as organic leather (Mersiowsky et al., 2001; Howard, 2002; Sivan, 2011; Avar et al., 2012;). Up to now, these materials have also notoriously lacked the quality, prestige, tactile properties, comfort and long-term durability of natural leather (Wang et al., 2014; Forgacs et al., 2016;). These materials are unable to perform as well as natural leather, neither mechanically, aesthetically nor texturally, and may even be more harmful to the environment than leather itself.The resultant premature product obsolescence, combined with fast-fashion culture, stimulates unnecessary waste. Plastic-based faux leathers, therefore, still have significant detrimental impacts on the environment (Petter, 2019; Pithers, 2019).

High-end, global brands, such as Stella McCartney and Nike, have already expressed significant interest in development of novel leather alternatives (Greene, 2016; Stella McCartney, 2020a). As an ethically and environmentally conscious brand, Stella McCartney completely refrains from animal product use (PETA, 2020a). The company has already collaborated with Bolt Threads, who have successfully developed an in vitro, protein-based alternative to silk, with comparable properties (Threads, 2019; Stella McCartney, 2020b). As a biodegradable and cruelty-free option, this is now preferred by the designer. Yet, a comparably sustainable and high-quality leather alternative is not currently available. Various plant-based substitutes (e.g. Pinatex, Frumat, Mylo) have started to enter the market, but production remains on a low scale (Carrara, 2018; Bolt Threads, 2020; “Pinatex,” 2020). Whilst these fabrics meet sustainability criteria, unlike plastics, they similarly cannot compare with the lustrous properties of genuine leather. As a result, designers remain limited in their options of commercially available leather alternatives. With high-end brands already encouraging further innovation though, other companies will be incentivised to compete and new materials should enter the market (Sharkey, 2019).

Perhaps a suitable approach to manufacturing a more sustainable leather-like material could be to engineer animal skin constructs within a laboratory (Jakab et al., 2019). The need for a more-suitable and sustainable leather substitute has coincided with the emergence of cellular-agriculture technologies, through which it is hoped that leather-like materials may soon be engineered in laboratories from collagen, instead of relying upon animal slaughter.

Engineering of human skin tissue substitutes within laboratories, using cell culture technologies, has already been researched for wound treatment (Horch et al., 2005; MacNeil, 2008; Subramanian et al., 2011; Chouhan et al., 2019;). In theory, principles of this methodology could be applied to the cultivation of an animal leather-like material. If eventually successful on a large scale, the fabric could attain acceptance by designers as a leather alternative in the future. Despite the potential for the emergent materials to offer significant design, sustainability, environmental and ethical benefits over real leather, much more time, effort and resource need to be focussed on the research to provide an improved base from which the technology can become sufficiently-well developed. In vitro engineered leather remains a niche research area. In this paper, a literature review discussing genuine leather manufacture and the possibility of a laboratory-grown alternative is presented. As a multi-disciplinary subject, the intended audience of this paper spans across researchers and practitioners in the textile, biomaterial and sustainability sectors. Relative ethical and environmental consequences of each method are assessed. The likelihood and impacts of scaling up laboratory production to meet current high leather demand also require consideration. Although sparse, any existing efforts in the field shall be discussed and suggestions for strategy adaptations made. As a tissue engineered leather substitute is not yet commercially available, there must be drawbacks in previous innovations and new developments still required. This paper acts as the first extensive review of literature with respect to the topic. By highlighting gaps in existing research from the currently available evidence, the article may guide the priorities of researchers within the field. The review also intends to increase general awareness of a laboratory engineered leather-like material's potential, so that acceptance by the public is readier in the future.

Improvements of that kind are still required though, as are more-efficient methods of controlling the collagen network structures created; allied research ought to focus upon increasing cell culture efficiency using, for example, specialised bioreactors. This issue of Textile Progress provides both a compilation and critical review of the existing literature relating to leather substitutes to enable the highlighting of such gaps in research to prompt further study and to increase awareness about the potential advantages of in-vitro alternatives to animal products like leather. The idea is also that with the spread of improved knowledge about the advantages of the emerging products, steps may soon be taken to help the wider public to become more aware, create demand and be better prepared to accept them, as soon as technological progress has reached a stage which allows market entry.

2. Leather

This section will present the literature describing current and long-established manufacturing processes in the leather industry. Shortcomings of these methods will be discussed, and potential improvements suggested for future work. It will begin with explaining the structure of animal skin and how the raw hide is processed before tanning. It will then focus on the tanning and finishing of leather, concluding with leather characterisation techniques.

2.1 Leather Manufacture

Genuine leather manufacture has principally not changed for centuries (Bosnic et al., 2000; Hüffer and Taeger, 2004). Some natural chemicals have been substituted for synthetics in modern manufacture, but the goal is consistent: animal skins are preserved in their supple, hydrated form as leather (Watt, 1906).

This section gathers literature describing current manufacturing processes in the leather industry. Shortcomings of these methods are discussed, and potential improvements suggested. The general steps required to produce finished leather from a raw animal hide are provided in *Figure* 1 (Sharphouse, 1995b; Bacardit et al., 2015; Laurenti et al., 2017). From this outline, it is apparent that leather manufacture is a complex process, with multiple procedures taking place at each stage. The key method of preserving animal hides against putrefaction is tanning, but many preparatory and finishing steps are also required. Clearly, leather manufacture as it stands is a work, resource and time-consuming process. In this section, each of the main production stages shall be considered in more detail.

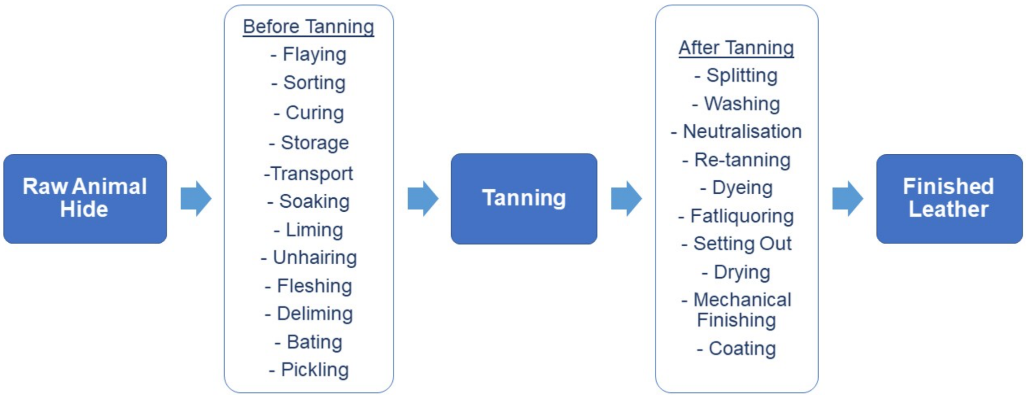

Figure 1: Schematic outline of the typical steps required during leather manufacture.

2.1.1 The Structure of Animal Skin and Leather

Animal skin *in vivo* performs a variety of functions that allow continual operation of internal organs. Skin maintains the structure of the body; provides protection against the elements and microorganisms; regulates temperature/fluid intake and enables sensory response (Subramanian et al., 2011). Skin also has the ability to heal itself. The major component of live animal skin is water, at approximately 64 % (Sharphouse, 1995c). This is followed by proteins at around 33 %. Of these proteins, the most abundant is collagen, which constitutes the main nanofibrous structural network. Collagen has many forms, but the predominant type in animal tissue is Type I (Parry and Craig, 1988). Individual polypeptide chains have a repeating X-Y-Gly amino acid sequence, where X is often Proline and Y Hydroxyproline in mammalian tissue (Grassmann et al., 1957). The presence of Glycine as every third amino acid sterically enables rearrangement of individual collagen strands into a right-handed triple helix (Ramachandran and Ramakrishman, 1976; Siggel et al., 2007). Meanwhile, X and Y stabilise the helix via hydrogen bonding between peptide chains (Ramachandran and Kartha, 1954; Piez et al., 1963; Ramachandran et al., 1973). Structural rigidity is attained through hierarchical formation of three-dimensional (3D) collagen fibres, from fibrils of multiple triple helices (*Figure 2*) (Hall et al., 1942; Bear, 1944; Gross and Schmitt, 1948). Collagen fibrils and fibres are crosslinked by hydrogen bonding between triple helices. These interactions are indirect, via water molecules when skin is hydrated (Bailey and Paul, 1998).

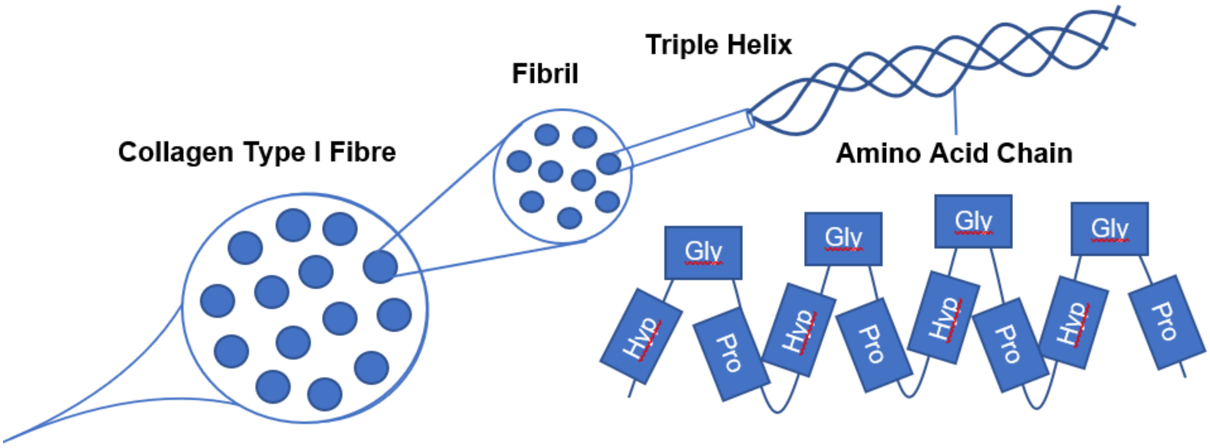

Figure 2: Diagrammatic representation of the hierarchical, fibrous structure of collagen I protein within animal skin tissue.

Animal skin is defined by a multiple layer composition (*Figure 3*) (Sharphouse, 1995c). The epidermis is the outermost layer of skin, exposed to the surrounding environment. This layer contains protective, dead, keratinous cells that are removed during leather processing to attain a uniform surface (Covington, 2011b). Below the epidermis is the dermis. Only the dermal layers of skin are applicable to the final composition of leather. The upper grain layer has a dense network of fine collagen fibres with a high angle of weave relative to the skin surface. Whilst most of the skin is composed of Type I collagen, the grain layer also contains Type III (Covington, 2011c). Type III collagen fibres are finer and weaker, but provide flexibility, as well as the distinctive surface texture of leather. The most expensive, premium leathers are full grain grade (*Figure 3*) (Dalgado, 2019). Only high-quality hides are used for full grain leathers, as any imperfections on the outer surface (e.g. scarring, blemishing) remain visible (Walker et al., 1990; Yeh and Perng, 2001). Generally, the highest quality part of a hide is the back, as less superficial damage is likely to occur when an animal is alive (Tucker, 2017). Surface area and thickness of sufficiently high-quality leather for commercial use is limited by animal skin size. Additionally, there is wide scope for variation in hide quality, due to factors such as climate, animal age and upbringing (Hadley et al., 2005; Abebayehu and Kibrom, 2010). Full grain leather is hence reserved for applications where thick leather with exceptional durability is vital, such as saddlebacks (Tucker, 2017). Top grain leather has the hide’s outer surface removed, so imperfections are no longer visible in the final material. Top-grain leather is generally used for high-end fashion goods. Elastin fibres within the grain enable live skin to stretch. Top grain leather has the hide’s outer surface removed, so imperfections are no longer visible in the final material. (Covington, 2011b). The lower corium of the dermis comprises of less densely woven, but thicker collagen fibres, with a smaller angle of weave (Haines and Barlow, 1975). As well as collagen, the overlapping region between the grain and corium in live skin contains hair follicles, blood vessels and sweat glands (*Figure 3*). A veiny surface texture is avoided in traditional leather manufacture by bleeding animals thoroughly after slaughter (Covington, 2011b). Native skin contains functioning cells (e.g. fibroblasts, endothelial) throughout the layers (JJ. Tancous, 1969). Beneath the dermis lies fatty flesh tissue that is, like the cellular components, removed before leather manufacture. Split leather is comprised of the loosely woven, internal corium layer of skin, so these leathers are cheaper and suppler (*Figure 3*). Suede is a form of split leather that is abraded on the corium flesh side to give a soft nap. Bonded leather is the lowest quality material, often used in cheap furniture, that is composed of hide offcuts bound with polyurethane (Tucker, 2017).

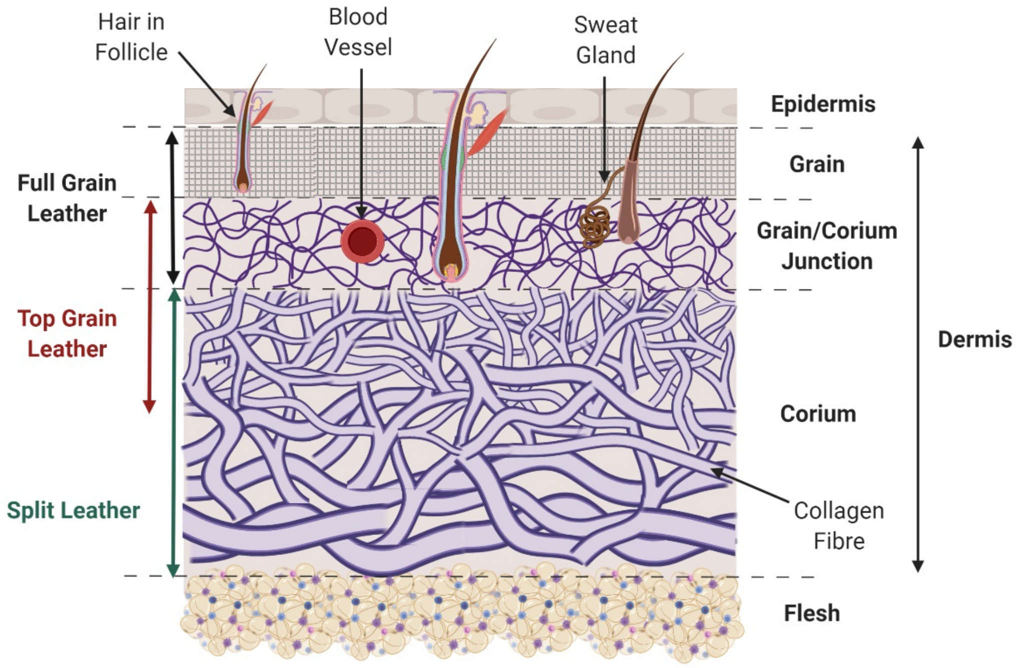

Figure 3: Diagrammatic representation of the structural layers within animal skin, where collagen fibres become gradually finer and more densely woven nearer the skin surface. Hairs are found in the top few skin layers and blood vessels in the grain/corium junction. Leather grades are approximately designated. Image created with BioRender.com.

Different animal hides are chosen for leather, according to attributes required in the final product. Their properties vary, depending upon the collagen network structure (Kelly et al., 2018; Naffa et al., 2019). Cow hides, for example, are thick (4-10 mm), with tightly woven collagen fibres, of large diameter, that promote durability (Dowling, 1955; Tulloh, 1961; Haines and Barlow, 1975; Kobayashi et al., 1999). Therefore, cow hides are preferable for leathers in hardwearing, shape retention applications, such as shoe uppers (Sharphouse, 1995e). Sheepskins, on the other hand, are thinner (1-2 mm), with a looser, finer collagen fibre network, so are softer and more flexible (Haines and Barlow, 1975; Butler and Head, 1993). They are more suitable for supple leathers, used in gloving for example (Sharphouse, 1995e). There is a restricted range of animal hides permitted for use in the leather industry. Commercialisation of exotic leathers, derived from reptile skins for example, is controlled, to protect endangered species. A lack of awareness has further confined the number of animal skin types utilised in the leather industry, often contributing to unnecessary waste (Karthikeyan and Chandra Babu, 2017; Sathish et al., 2017; Belay et al., 2019). Design scope with leather is, therefore, limited at present.

2.1.2. Raw Hide Processing Before Tanning

Prior to permanent preservation of animal hides during tanning, several preparatory steps are required (*Figure* 1). Immediately after removal from slaughtered animals, hides are trimmed during flaying, then sorted for quality. Off the carcass, animal skin is prone to degradation, since peptide bonds within collagen proteins are cleaved by bacterial enzymes (Lindner and Neuber, 1990). This putrefaction must be prevented quickly, to avoid skin protein structure damage, then consequent loss of hide quality and value (Didato et al., 2008; Gbolagunte and Hambolu, 2010). Preservation is via a combination of destroying active bacteria, limiting bacterial activity and preventing contamination. Often, large quantities of salt are used during hide curing (Wu et al., 2017). Alternative methods include drying; acidic solution treatment or bactericide application (Sharphouse, 1995f). Temporary preservation of raw hides is currently necessary during transport because animals are usually slaughtered in a different location to where they are permanently tanned.

In addition to the collagen framework utilised in leather, animal skin contains fatty flesh, keratin and non-structural interfibrillar proteins, such as globulins (Sharphouse, 1995g). Liming removes these components from hides in traditional leather manufacture. If untreated, the different protein structures would resist collagen tanning and the resultant leather would have an uneven finish. Typically, hides are pre-soaked in water, then an alkaline solution of calcium hydroxide and sodium sulfide initiates hydrolysis of hair and interfibrillar proteins (Covington, 2011d). Prior to tanning, the hide needs to be neutralised with acid, often sulfuric, during deliming. Further acid pickling attains the optimum pH for tanning. Care is taken not to leave hides at high pH for too long though, as this can allow hydrolysis of peptide bonds within collagen itself (Hofman et al., 2011). Prior to tanning, hides may also be softened during bating to improve tannin penetration.

2.1.3. Tanning of Hides

After lengthy preparation, raw animal hides are currently and traditionally, preserved permanently as leather through tanning. Hydrated collagen fibres are swollen, due to the free volume taken up by hydrogen bonded water molecules. The skin remains soft and flexible. Upon skin dehydration, collagen fibres shrink and eventually, helices become in close enough proximity to hydrogen bond directly with one another (Sharphouse, 1995h). A lack of free volume causes a dehydrated fibre network to be dense, stiff and inflexible. Skin surface area is also reduced. Tanning maintains a hydrated style of network structure, through bonding of collagen to tanning agents, instead of water molecules. Tanned hides retain flexibility and surface area upon drying (Covington, 2011e). A variety of tanning agents are available, depending upon the final leather properties desired (Sizeland et al., 2016). Vegetable tannins are extracted from natural, plant-based resources, such as mimosa bark or chestnut wood (Sharphouse, 1995h) These tans produce stiffer leathers, suitable for applications such as shoe soles, with a brown colour (Haslam, 1997; Spencer et al., 1988). According to China, C.R. et al., vegetable tannings have less and manageable environmental effects (Madhan et al., 2006; China C.R. et al. 2020). Traditionally, vegetable tans were the most commonly used in leather manufacture, yet man-made chemical agents are favoured in modern methods (Wilson, 1941b, 1941c; Covington, 2011f). Synthetic tannins (Syn-tans), mimic vegetable tannins, but they are artificially engineered to hydrogen bond with collagen in specific positions (Ammenn et al., 2015). Syn-tans can enable faster processing and manipulation of leather properties, such as hydrothermal stability, flexibility and light fastness, through controlled bonding (Covington, 2011g). However, these advantages are at the expense of tanning reversibility, material biodegradability and tan renewability.

Chromium (III) sulfate salts stabilise the collagen network of skin through coordination bonding. These are the most common tanning agents used in leather production today, due to the advantageous properties achievable (Chattopadhyay et al., 2012). Chromium salts enable production of flexible leathers, suited to applications such as shoe uppers, as less tanning agent is fixed to collagen than with vegetable tannins (Covington, 2011f). Chrome tanned leathers have a pale, blueish colour, which increases ease of subsequent dyeing, compared with dark coloured vegetable tanned leather (Covington, 2011h). Chromium salts have however, become an environmental concern upon entering tannery effluent (Section 2.1.5). Metal ions with lower environmental impact, like Aluminium (III), Titanium (IV) and Zirconium (IV), have been considered as replacements for Chromium (III) (Covington and Sykes, 1984; VanBenschoten et al., 1985). Unfortunately, their salts have weaker tanning ability, so Chromium (III) remains the principal leather tanning treatment (Dunhill et al., 1990). Chrome accounts for 90% of global tannery (Hao et al., 2023) and results in chromium-containing solid waste and wastewater (Li et al., 2020; Liu et al., 2022; Hao et al., 2023). Although, uptake of Chromium (III) can be more efficient in the presence of a catalytic metal salt with weaker coordination bonding, such as Aluminium (III) sulfate (Covington, 1986). Chromium waste in tannery effluent is hereby reduced.

2.1.4. Finishing of Hides After Tanning

Although tanning is the major procedure in converting a raw animal hide to leather, in this subsection we will be discussing the several finishing processes that can then take place (*Figure* 1). As described in Section 2.1.1, the tanned hide may be split into thinner sections, depending upon the grade of leather desired. Any residual tanning agents ought to be washed out of the hide and the pH neutralised before re-tanning if necessary. Tanning does not always achieve the final colour desired from leather, so an additional dyeing step may be needed. Following dyeing, a wide range of additional finishes can be carried out to achieve the final leather physical properties required. The most executed procedure is fatliquoring, whereby an oil is transported into collagen fibre bundles as an emulsion in water (Covington, 2011i; Sharphouse, 1995i). Upon leather dehydration, the oil can flow over collagen fibre surfaces and thus lubricate them to avoid too closer adhesion. A secondary consequence of this action is leather softening. After fatliquoring, leathers are set out, to achieve an even surface texture without creasing, then they are dried. A variety of mechanical finishes or coatings may subsequently be applied to alter leather properties. A particularly useful finish is reduction of collagen fibre wetting ability using hydrophobic polymers, to evoke water resistance (Hodder, 1995). Aqueous fluids can still flow between collagen fibres towards the surface of leather for evaporation. For example, rainwater is repelled by shoe leather, yet sweat also wicks away from feet. For these reasons, genuine leather is vastly preferred to purely polymeric alternatives for comfortable footwear (Bitlislp et al., 2005). Once final quality checks are made, complete leathers become commercially available to product manufacturers for the next stage in their life cycle. It is important to note, as highlighted in Table 1, that there are potential consequential pollutants in wastewater from post-tanning as not all chemicals are taken up during this process (Hansen et al. 2021a, 2021b). In the following subsection, we will be highlighting future challenges facing the industry when considering the full life cycle of leather and associated environmental aspects.

2.1.5 Life Cycle of Leather and Associated Environmental Impacts

We have highlighted previously, the traditional and current production processes associated with leather manufacture and begun to draw attention to the associated environmental impacts. In this section we will focus on the resource inputs and waste outputs across the current life cycle, presenting current and future advances in this area.

The entire life cycle of leather is split into three key stages: initial animal agriculture; leather manufacture itself and finally, the production of leather goods (Figure 4) (Joseph and Nithya, 2009). Throughout leather's life cycle, many resources are used; energy consumed and waste produced (Laurenti et al., 2017). The leather manufacturing stage includes the steps outlined in *Figure* 1. The major resource needed during this phase is water, namely for rinsing and chemical solutions. Often, processing takes place in locations with a limited supply of clean water, such as India. Water does not constitute the final material, so all that is used goes to waste. Another problem is that water becomes a carrier for other waste products, which can hinder reuse (Gutterres et al., 2008). These can contaminate water sources if effluent is insufficiently treated before disposal. Temporary hide preservation following animal slaughter, for example, introduces salts to wastewater (Section 3.2). Sodium and chloride ions are particular concerns (Wu et al., 2017). Salts are also formed during neutralisation reactions. Several studies report that ion contents in waterways near tanneries are above tolerable limits for drinking (Cooman et al., 2003; Mondal et al., 2005; Brindha and Elango, 2012; Zhou et al., 2012; Ilou et al., 2014; Kanagaraj and Elango, 2016). Concentration of ions within soil can also reduce crop fertility and therefore, productivity (Karunyal et al., 1994). By-products of protein hydrolysis during liming, unhairing and fleshing steps include ammonia and its ammonium salts (Sharphouse, 1995g). These too can be harmful to human health and toxic to aquatic life if allowed to enter waterways (Randall and Mandy, 2004; Camargo and Alonso, 2006). Extremes in pH during hide preparation for tanning pose a health risk to workers. Proteolytic enzymes are a less harmful, pH neutral option for protein degradation (Taylor et al., 1987; Dettmer et al., 2013). There is, however, a greater risk of collagen breakdown with enzymes, so conditions need to be more strictly controlled (Alexander, 1988; Sivasubramanian et al., 2008; Valeika et al., 2019).

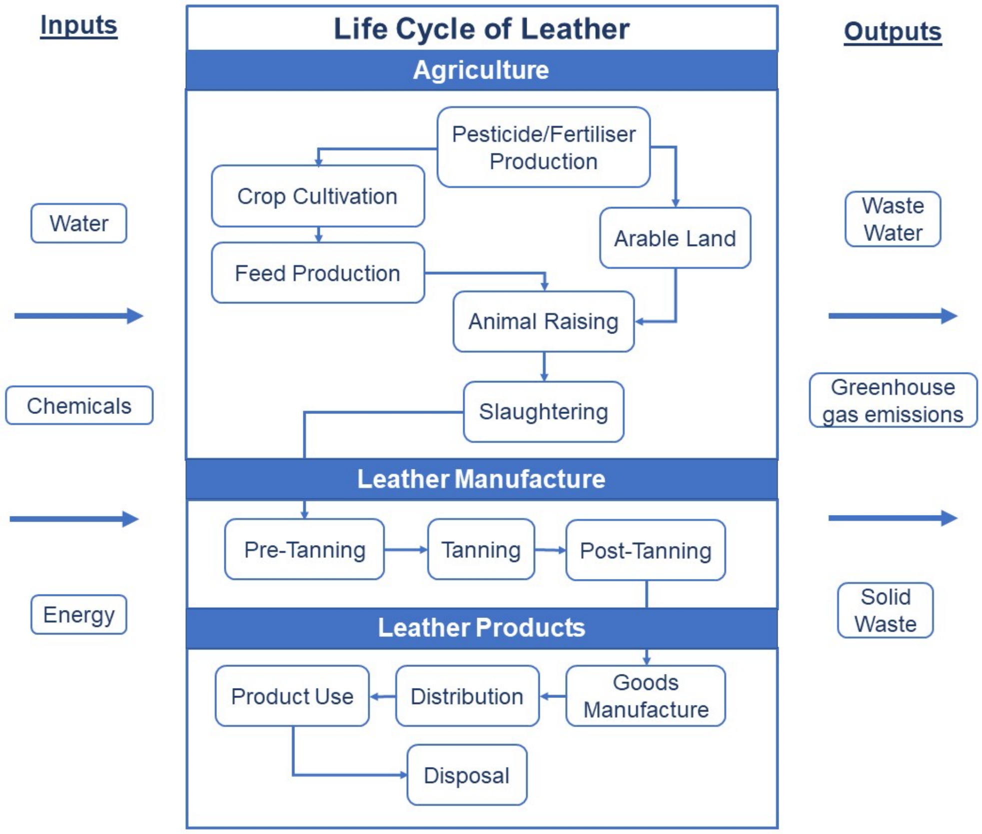

Figure 4: Diagrammatic representation of the typical life cycle for leather, including resource inputs and waste outputs.

The major source of chemical contaminants in leather production is the tanning process (Section 2.1.3) (Joseph and Nithya, 2009). Table 1 compiles the broad range of chemical contaminants in leather production.

Consequential Pollutants from Preparatory Processes and Procedures	
(Saxena et al., 2020)	• Sulfuric acid/sulfide, • mono or disodium phosphate/polyphosphate, • ammonium salts.
(Dixit et al., 2015)	<u>Volatile Organic Compounds</u> • Ammonia • Sulphides
Chemical Contaminants from Tannery Effluent (TE)	
(Saxena et al., 2020)	• Phosphate, • Nitrate, • Sulphate, • Phenol, • Blend of noxious organic and heavy metal contaminants
(Dixit S et al., 2015)	• Chromium (Cr), • Vegetable tannins,

	• Syntans, • Phenolics, • Azodyes, • Pesticides, • Sulphonated oils, • Polychlorinated biphenyls (PCBs), • Nonylphenols (NP), • Phthalates.
Potential Consequential Pollutants in Wastewater from Post-tanning	
(Piccin et al., 2016; Ortiz-Monsalve et al., 2019; Hansen et al., 2020; 2021a)	• Deacidulants, • Synthetic and natural fatliquoring agents, • Surfactants, • Synthetic and natural retanning agents, • Dyes, • Chemical auxiliaries, • Acids.

Table 1: The broad range of chemical contaminants in leather production (Dixit et al 2015, Saxena et al 2020, Hansen et al. 2020; 2021a)

Tanning agents are generally used in excess and large quantities remain in wastewater (Legesse et al., 2002; Chattopadhyay et al., 2012; Bhargavi et al., 2015). Sulfate and related sulfide ions from mineral tanning salts and acids, used to optimise the pH for tanning, reduce the oxygen content of water (Sharphouse, 1971). The reduction in oxygen content in the water has been reported to be harmful to aquatic life in freshwater systems (Randall and Mandy, 2004; Camargo and Alonso, 2006). Chromium in the most common tanning salts is usually in the stable Chromium (III) form, but can be oxidised to the toxic Chromium (VI) state in soil (Bartlett and James, 1979). Chromium (VI) can persist in soil, if reduction capacity is exceeded (Sreeram and Ramasami, 2003). There is evidence that chromium from leather tanneries has entered nearby waterways (Brindha and Elango, 2012; Zhou et al., 2012; Kanagaraj and Elango, 2016). Water polluted with chromium is unsafe for human consumption and damaging to aquatic life, because Chromium (VI) can enter biological cells through anionic transport proteins (Venugopal and Reddy, 1992; Singh et al., 1998). As an oxidising agent, the ion can then act as a carcinogenic mutagen (Green, 1985; Guida et al., 2019). The health risk to tannery workers is generally high, because, as seen in *Figure 5*, the largest scale tanneries are often located in poorer countries with less stringent safety regulations (Shankar, 2014). When observing global production of leather, 60% is held by developing countries, led by China and then India (Leather Goods Market Growth & Trends, 2021; De Ponte et al., 2023). Studies have already confirmed that tannery workers exhibit heightened cancer rates (Rastogi et al., 2007; Balachandar et al., 2010). Although, the general public residing near tanneries also have their health put at risk upon exposure to contaminated water. Additional toxic and non-biodegradable chemicals, such as synthetic, non-biodegradable dyes, are used throughout leather finishing (Section 2.1.4). These can leach into water supplies as well and cause further environmental problems (Laurenti et al., 2017; Tasca and Puccini, 2019).

Figure 5: An example of leather processing taking place at Chouara tannery in Fez, Morocco. This is the largest tannery in the city and has continued to use only manual labour since medieval times (Patowary, 2014). These traditional techniques offer inadequate protection from toxic chemicals to workers' health and the environment. Photo credit: (Nash, 2014) Photo credit: 'Fes Morocco Tannery - sept 2014 - 04' by andynash, licensed under CC BY-sA 2.0. To view a copy of this license, visit https:// creativecommons.org/licenses/by-sa/2.0/?ref=openverse.

Due to such significant environmental and health hazards, effluent from leather manufacturing plants have strict limits imposed upon harmful chemical content prior to disposal. The maximum acceptable quantity of chromium in wastewater from a tannery is 2 ppm (Sreeram and Ramasami, 2003). Without satisfactory waste processing in place, this value can be as large as 2500 ppm (Ramasami, 1996). In less economically developed countries, these effluent treatment procedures may not yet be fully established (Bosnic et al., 2000; Konrad et al., 2000; H. L. Paul et al., 2013). Short-term profits that boost the economy are likely to be of higher priority to tanneries in such regions (Chowdhury et al., 2018). Non-putrescible waste from leather manufacture is not readily treatable chemically, even with advanced facilities in place. Degradation of organic matter to biogas by anaerobic enzymes is a novel, more sustainable possibility (Agustini et al., 2017; Priebe and Gutterres, 2017).

Considering the entire life cycle of leather (*Figure* 4), further environmental concerns are presented at the initial animal agriculture and final leather product manufacture stages (Joseph and Nithya, 2009; Laurenti et al., 2017; Tasca and Puccini, 2019). Traditionally, leather is manufactured as a by-product from the meat industry (Wilson, 1941a). However, only around 25-30 % of these wet-salted hides actually become leather (Sharphouse, 1971). The rest of the original material, including water, flesh, hair, salt and surplus chemicals, is wasted. The exception being in fellmongering of sheepskins. Often animal hides for leather are sourced from countries with poorly enforced animal rights and waste management laws (PETA, 2020a). In order to meet high demand and increase profit, some of these animals are in fact intensively factory farmed, purely for their skins (Van-Eelen et al., 2006; PETA, 2019). The proportion of waste can be even higher from such sources, since there is an increased risk of disease outbreak amongst large numbers of animals reared in close proximity (Van-Eelen et al., 2006). The food industry has already identified the unsustainability of animal agriculture as a significant concern for meeting future demand as the global population rises (Pimentel and Pimentel, 2006; Breemhaar and Post, 2019). Similarly, demand for a material with comparable properties to leather is still set to increase as populations and wealth grow. Crop cultivation uses pesticides and fertilisers to feed the animals that will eventually be slaughtered. Pesticides can accumulate to toxic levels in organisms found higher up food chains; whilst fertilisers risk soil acidification, groundwater pollution and eutrophication (NSW Government: Department of Primary Industries, 2019). Intensive animal farming and slaughter also present risks to human health through disease transmission and antibiotic resistance (Vein, 2004; Elfenbein and Kolbeck, 2018; Ben-Arye and Levenberg, 2019). Large areas of arable land, energy and water supplies are also required for livestock upbringing and feeding. The majority of calories consumed by livestock is wasted on their metabolism and production of less useful tissues (Ben-Arye and Levenberg, 2019). Meanwhile, crops currently used to feed farm animals could instead address global famine (Vein, 2004). Forests are often cleared to create arable land, but there is a finite area available on the planet, which is unable to sustain rising animal product demand (Van-Eelen et al., 2006; Tuomisto, 2019). In combination with methane release upon fermentation by ruminant livestock, there is significant contribution to the greenhouse effect (O'Mara, 2011). Globalisation meanwhile, has led to the manufacture and export of leather goods across the world, particularly in the age of 'fast-fashion' (Tokatli, 2008). Each stage of leather production can occur in a different country (Sharphouse, 1995b). Transportation consumes energy in the form of unsustainable fossil fuels, further contributing to the greenhouse effect and climate change (Stern, 2007). The additional energy consumed throughout leather's life cycle, to power farm, factory, laundry and waste disposal machinery, leads to a large carbon footprint overall (Joseph and Nithya, 2009; Azzouz et al., 2017; Chen et al., 2019; Tasca and Puccini, 2019).

At the end of leather's life cycle, disposal is an important consideration. Leather can have a lower environmental impact at this stage than oil-based synthetic alternatives, as organic material is biodegradable (Mersiowsky et al., 2001; Howard, 2002; Ferreira et al., 2010; Sivan, 2011). Although, the large quantities of hazardous, solid, sludge wastes produced during manufacture are generally disposed of in landfill (Agustini et al., 2017, 2016). Leather's biodegradability is also subject to what chemicals were applied during processing. Synthetic tanning agents, for example, slow leather degradation (Ollé et al., 2011; Qiang et al., 2012; Guida et al., 2019). Sulfated paraffin oils from unsustainable crude oil, used during fatliquoring, are difficult to separate from leather for recycling or biodegradation (Tasca and Puccini, 2019). Toxic, non-biodegradable surfactants used to generate the oil in water emulsion pose health and environmental risks (Kowalska et al., 2019). As with fatliquors, polymeric coatings within bonded leather or water resistance finishes are sourced from unsustainable crude oil and inhibit material recycling or biodegradation (Tasca and Puccini, 2019).

Efforts have been made to increase the sustainability of leather's life cycle. Examples include: improved treatment of tannery effluent to remove toxic chemicals (He et al., 2005; Tahiri and De La Guardia, 2009; Vedaraman and Muralidharan, 2011); sustainable preservation of hides (Kanagaraj et al., 2001; 2005; Sundar et al., 2019; Vedaraman et al., 2016; Kanagaraj et al., 2020; Tu et al., 2022) selective breeding of animals for optimum hide size and quality (Zapletal, 1997; Vein, 2004; Vale, 2010); identification of less harmful alternatives to reagents (Kolomaznik et al., 1996; Aravindhan et al., 2004; Saravanabhavan et al., 2004; Fathima et al., 2006; Rao et al., 2008; Jiang and Zhao, 2014); enzymatic and eco-benign soaking processes (Dettmer et al., 2012; George et al., 2014; Ma et al.

2014; Kowalska et al. 2019; Kanagaraj et al., 2020) sustainable dehairing of skins/ hides (Kanagaraj et al., 2020) via an oxidative process (Kanagaraj et al., 2016) and utilisation of waste products (Brown et al., 1996; Rangel-Serrano et al., 2003; Çolak et al., 2005; Yilmaz et al., 2007; Guo-Tao et al., 2013; H. Paul et al., 2013; Vedaraman et al., 2016). However, the leather making process is principally inefficient and improvements may only be to a finite extent. When brands have made promises to consumers in the past about provision of more eco-friendly leather materials, they have generally failed to deliver, despite significant financial investment (Givhan, 2015). This is no longer a viable option, as pressure, from both customers and sustainability regulation bodies, is rising (Brugnoli, 2017). Looking longer term, sustainability issues with animal agriculture may convert people to plant-based diets or cultured meat alternatives (Chriki and Hocquette, 2020). Consequently, the source of hides that the leather industry depends upon now, could dwindle. In case of this eventuality, an entirely novel manufacturing process must be considered. Otherwise, the rising demand for materials with leather-like properties will not be met, as existing leather alternatives are also unsuitable (Dal et al., 2019b).

2.1.5 Leather Characterisation Techniques and Standards

After production, the finished leather may be characterised using a variety of techniques. This may be to ensure it is fit for purpose, to ensure quality, to assess product development, safe consumption or to support sales and marketing of the product. In this section, we will highlight some examples of current leather characterisation techniques used within a standard, with examples listed in Table 2.

Before any chemical, physical, mechanical or fastness standard tests are carried out, leather specimens must be prepared and condition appropriately (ISO 2418:2023).

In Section 2.1.1, surface texture of leather was noted as one key parameter to determine the quality of the hide. Any remaining imperfections on the outer surface, such as scarring or blemishes, were noted as undesirable. Electronic techniques can be used to assess dry and wet leather surface (ISO 1907:2023) but further surface properties such as coating thickness (ISO 12186:2011) and resultant flex resistance (ISO 5402-1:2022) can be further assessed. Further testing can be carried out specific to the product application. An example for flexibility, would be assessing the impact of finish on flex of upholstery leather (ASTM D2097 - 03-2023).

Physical and mechanical tests can be carried out to assess surface area and thickness to ensure appropriateness for commercial use (ASTM D1813 - 13-2023). This is important to assess as Section 2.1.2. noted that there may be variations in the quality of the animal skin due to climate, animal age and upbringing. Tensile (ISO 3376:2011) and tear strength (ASTM D6077 - 16-2023) test standards can be carried out to ensure the chosen leather hides have the required durability for the end product. Together with upholstery leather, there are specific standards for leather applications such as shoes and apparel, where physical and mechanical properties, such as water vapor permeability are required (ISO 14268:2023). Leathers used in garment manufacture must adhere to the performance requirements noted in BS 6453:1984. This includes suede leather, simulated grain leathers and wool sheepskin.

Alongside physical and mechanical testing of leather specimens, there are also a variety of chemical and fastness standards that can be used. Examples include, but are not limited to, assessing azo colourants in dyed leather (ISO 17234-1:2020) quantitative analysis of tanning agents and colour fastness. As highlighted in Section 2.1.3 by testing specimens of the leather and achieving the desired result throughout the material, ensures even processing has been carried out from raw hides through to finished leather.

Standard Number ID	Title
ISO 2418:2023	Leather - Chemical, physical, mechanical and fastness tests - Position and preparation of specimens for testing
ISO 1907:2023	Leather - Measurement of leather surface - Electronic techniques
ISO 12186:2011	Leather - Physical and mechanical tests - Determination of surface coating thickness.
ISO 5402-1:2022	Leather - Determination of flex resistance - Flexometer method
ISO 14268:2023	Leather - Physical and Mechanical Tests - Determination of water vapor permeability
ISO 3376:2011	Leather. Physical and mechanical tests. Determination of tensile strength and percentage extension.
ISO 17234-1:2020	Leather - Chemical tests for the determination of certain azo colorants in dyed leathers. Determination of certain aromatic amines derived from azo colorants.
ISO 14088:2012	Leather - Chemical tests - Quantitative analysis of tanning agents by filter method.
ISO 7906:2022	Leather - Tests for colour fastness - General principles of testing.
ASTM D6077 - 16(2023)	Standard Test Method for Trapezoid Tearing Strength of Leather
ASTM D1813 - 13(2023)	Standard Test Method for Measuring Thickness of Leather Test Specimens.
ASTM D2097 - 03(2023)	Standard Test Method for Flex Testing of Finish on Upholstery Leather
BS 6453:1984	Specification for performance of leathers for garments

Table 2 - Examples of Test Standards for Leather and Leather-Like Materials

3. Leather Alternatives

Whilst the focus of this review is the production of leather-like materials by cellular agriculture, it is important to note that there are, and have been, other approaches. The examples provided in this section are not exhaustive; they aim to highlight the challenges associated with replicating performance, mechanics and aesthetics of 'real' leather. Furthermore, they draw attention to considerations required towards the feedstock of the material and provide insights of drawbacks to consider in future work.

3.1 Historical examples

The concept of producing an alternative to leather is nothing new. According to Kanigel, R. (2010) as early as the 14th Century we have been seeking alternatives, by coating fabrics in oil, and later in the 1800s coating cotton in wax to produce a 'leatherette' material for book binding.

In the 20th Century, DuPont and others began actively seeking man-made alternatives to compete (and surpass) the natural competitor. These materials are referred to as poromerics (Gooch, J.W. 2007). These materials are defined as '…water vapour-permeable leather substitutes…' by D.A. Littler and A.W. Pearson (1972).

Commercially available products emerged, carefully marketed to avoid the use of the term leather. These included, but are not limited to, Fabrikoid (used for the seats of Model T-Ford cars) Corfam and Quox (Kanigel, R. 2010).

According to Kanigel, R. (2010) Corfam was a breathable and homogeneous substitute. Aesthetically, the grains on the surface of the material produced resembled that of leather. Yet they claim, mechanically, it was inferior and lacking absorbency. They highlight that consumer reports at the time noted the 'blandness' of Corfam's uniformed appearance and enduring 'newness'. Furthermore, consumers found the aging of leather appealing. Lastly, the author attributes Corfam's downfall to the slow adaptability of the fabrication process, to fast, changing fashion and consumer needs, which experienced, skilled tanners could respond to (Kanigel, R. 2010). All noteworthy points to consider when designing 21st Century alternatives.

3.2 Natural or synthetic feedstocks

With the current focus on sustainability, it is important to note the feedstocks in historical and current alternatives to highlight and explain the challenges associated with incorporating them. If we classify textile materials according to origin, those deriving from natural resources include cotton and flax (cellulosics) silk and wool (proteins) and man-made synthetic fibres include polyester, polyamide, lycra and acrylic fibres deriving from fossil fuel feedstocks. There are also man-made fibres deriving from natural polymers, such as viscose (rayon) lyocell, ramie and bamboo.

When referring back to poromeric examples provided in section 3.1, Fabrikoid, according to Meikle, J.L. (1995) was a nitrocellulose product produced by '…coating rolls of cotton fabric with a 'dope' or 'jelly' of nitrocellulose dissolved in castor oil, alcohol, benzene, and amyl acetate…'.

Later in the mid 20th Century, composite materials such as Corum were made from urethane polymers and polyester fibres (Gooch, J.W. 2007). It was produced from a punched nonwoven material, its structure aimed to replicate the collagen bundles of skin (Kanigel, R. 2010).

Today, there are growing concerns of using fossil fuel-based materials in leather substitutes, (Section 1). Bielak and Marcinkowska (2022) explain that '…synthetic materials imitating natural leather…are most often obtained by applying a layer of plastic (e.g., polyvinyl chloride) onto a textile carrier (e.g., fabric) or by impregnating a textile carrier with synthetic resins (e.g., polyurethane) and resin coagulation in the carrier…' as shown in Figure 6.

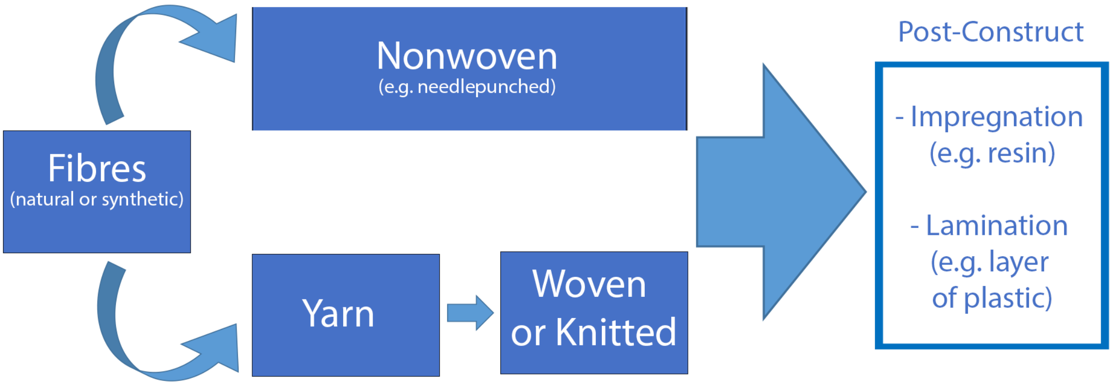

Figure 6: Schematic displaying example of steps to creating leather-like substitutes Bielak and Marcinkowska (2022)

When considering environmental sustainability, research and industry are reverting back towards natural resources. This includes, but is not limited to, the use of mycelium and microbium cellulose as a renewable resource (Peng et al. 2023). Some are further combined with woven, knitted and nonwoven materials to produce composite materials like their predecessors. Researchers today continue to produce new leather alternative materials that echo the efforts of the historical predecessors. Basak et al. (2022) for example, document the performance and mechanical properties of a new textile composite, created with a needlepunched nonwoven from ramie fibres coated in natural rubber. There are four main approaches to producing flexible fungal materials (FFMs) which are Wild Basidiocarp Foraging (WBF), Liquid-State Fermentation (LSF), Liquid-State Surface Fermentation (LSSF), and Solid-State Fermentation (SSF) (Gandia et al, 2021). Paper-like materials created via LSF have already been reported for appropriate use as biomimetic fungal scaffolds in human tissue engineering, and pure mycelium foams for biocompatible cellular scaffolds for biomedical applications (Pelletier et al., 2019; Narayanan et al., 2020; Gandia et al., 2021;).

According to Li et al. (2023) whilst fungal mycelium has shown great promise in studies, the versatility and engineerability is limited due to the two optional final production stages. These are either the process of heat-killing the living cells in the engineered living material or relying on the co-culture with a model organism for functional modification (Birnbaum, et al. 2021; Gandia et al., 2021; Jones et al., 2021; Yousefi et al., 2021; Li et al., 2023).

3.3 Environmental concerns and challenges

Despite the current efforts to mitigate environmental impacts, Hildebrandt, Thrän and Bezama, (2021) highlight through the life cycle impact assessment of three leather alternatives the importance of enhancing the durability of these substitutes and the use of low-impact coating systems and impregnation agents. This highlights the ongoing challenges with coated or impregnated composite alternative materials compared to animal leather. They also highlight the need to integrate best practice interventions, including feedstock supply as mentioned in Section 3.2 and end of life recyclability and degradability (as mentioned in Section 1). It is likely that as more commercial alternatives to bovine leather enter the market, researchers will continue to carry out life cycle assessment to determine if the alternatives meet the future challenges and concerns faced by the fashion industry. This is shown in the work by Williams, E., et al. (2022) who carried out life cycle assessment of MycoWorks' Reishi™ against bovine leather.

3.4 Characterisation of Leather Alternatives

In section 2.1.5. traditional and current methods of characterisation of leather (and leather substitutes) have been presented. In this section, the aim is to highlight that research and development into leather alternatives, apply these same standard tests to assess their performance and also to compare against leather. For example, Basak et al. (2022) assessed the performance properties of the flexural composite mimicking natural leather, by carrying out testing such as '…abrasion resistance, tensile strength, tear strength, puncture resistance, permeability, porosity and dynamic loading and recovery…compared with wet blue (chrome treated) goat leather…'. It is important to note, that these standard tests would continue to be applied to future developments, and perhaps influence any new testing observed.

4. Skin Tissue Engineering

This section will highlight the current design and use of lab-engineered skin tissue for medical applications. It will continue by noting the limitations as a substitution for human skin (sensory and regulation functions, for example). We will continue by suggesting and explaining how laboratory synthesis could follow principles from tissue engineering, but non-immunogenicity and complete skin functionality will not be necessary, as this tissue will not be implanted.

4.1 Overview of purpose and scope

Laboratory-engineered skin tissue was developed initially as a substitute for human skin to repair wounds due to the low availability of suitable donor tissue (Kirsner et al., 1998). Without engineered tissue, treatments would depend upon split or full-thickness skin grafts (Seal et al., 2001). Another source of skin tissue was required to effectively treat more patients and more significant wounds.

4.2 Scaffold use in medical applications

Up to now, engineered skin tissue remains almost exclusively implanted into medical patients for wound healing applications (Akter et al., 2016). In this role, artificial tissue must effectively mimic native tissue and be non-immunogenic upon implantation (Horch et al., 2005). Other smaller scale applications include skin biology research and trialling cosmetic product safety as an alternative to animal testing (MacNeil, 2007). The main drawback of tissue engineered skin is that it cannot currently function as complete, healthy skin (Akter et al., 2016). Substitutes lack the complexity of real skin. For example, they cannot perform sensory or regulation functions without the nerves, blood vessels, hairs, glands or pigments of genuine skin (Supp and Boyce, 2005). Instead, tissue engineered constructs tend to mimic structural elements of skin, then stimulate growth factor production around wounds to aid skin regeneration. Similarly, leather only contains the structural collagen components of native skin (Section 2.1.1). Laboratory synthesis could, therefore, follow principles from tissue engineering, but non-immunogenicity and complete skin functionality shall not be necessary, as this tissue will not be implanted. This could enable a simpler and more probable application of tissue engineering technologies (Jakab et al., 2019).

Skin has multiple layers, and its major structural component is the 3D, nanofibrous, collagen network (*Figure 3*). Engineered skin substitutes ought to imitate this structure, so that comparable material properties are attained (Venugopal et al., 2008). Tissue engineering involves seeding cells onto a suitable scaffold material. Additional nutrients and growth factors are then required by the cells for proliferation and product secretion. On a larger scale, cells may be grown in bioreactors or built-up layer-by-layer in culture vessels. If scaffolds are employed, they require high porosity for cell and nutrient infiltration (Yang et al., 2001). The main purpose of a scaffold is to direct the growth of seeded cells (e.g. fibroblasts) and the production of cellular products (e.g. collagen) (Hutmacher, 2001). For this, it is preferable for scaffolds to have a large surface area for cellular interaction. Scaffold topography should mimic that of natural extracellular matrix (ECM), in order to replicate real tissue structure in the final construct (Jayarama Reddy et al., 2013). Scaffolds may be composed of naturally extracted materials, like collagen itself, or synthetic polymers (Telemeco et al., 2005; MacNeil, 2008; Ghodbane and Dunn, 2016). Polycaprolactone (PCL) and Poly (lactic-co-glycolic acid) (PLGA) polymers are already approved for human implantation by The United States Food and Drug Administration (FDA) (Mansour et al., 2010). Although, for an *in vitro* engineered leather substitute, there could be more options, as the material is not for implantation. Depending upon biocompatibility, synthetic scaffolds may require additional surface functionalisation to improve cell adhesion (Subramanian et al., 2011). Fibrous scaffolds are favoured, as they inherently have high porosity, interconnectivity and surface area for cell attachment and infiltration (Senel-Ayaz et al., 2018). Properties, such as pore size and shape, are also readily controllable with this type of scaffold. Various techniques are employed to produce scaffolds, including particle leaching; freeze-drying;

high pressure gas expansion or electrospinning (Jayarama Reddy et al., 2013). Fibrous textile fabrics also have the inherent porosity needed to support cell and nutrient infiltration (Senel-Ayaz et al., 2018). Biocompatible, microscale fibres within such scaffolds can direct cell orientation and growth (Moroni et al., 2008).

5. Laboratory-Engineered Leather Substitutes

Under the following headings we will introduce the reader to current substitutes explaining, with the use of Figures, key current processes and strategies for producing leather substitutes in the laboratory in future.

5.1 Overview of current small scale commercial approaches

Despite the potential of engineering leather-like materials in a laboratory, this remains currently a very novel and unique area of research. There are various possible reasons for this. Research into skin tissue engineering in general has so far not been considered very profitable, due to the relatively low number of skin casualties in the West for treatment (MacNeil, 2007). Leather is a material that is consistently in high demand, so perhaps this new application could encourage further funding for skin tissue engineering research (Koppany, 2004). Perhaps, up until recently, synthesis of a more sustainable and ethical alternative to leather has simply not been a high enough priority. Current leather manufacturing processes are well established; profitable; and many people rely upon the industry for their livelihoods (Covington, 2011a). The industry is, however, unlikely to be capable of keeping up with demand in the future, so it is important to consider novel manufacturing processes now (Dixit et al., 2015). Of the groups that have already undergone research into laboratory engineered leather-like materials, Modern Meadow, a USA company, are currently the most active (Modern Meadow, 2020).

5.2 Technologies

5.2.1 Strategies and considerations

At present, various strategies have been explored during the development of leather-like materials within a laboratory, as shown in Table 3.

Company (Location)	Technology and Strategy
Le Qara (Arequipa, Peru)	Undisclosed microorganisms fed with plant residues are fermented to produce a biopolymer. The biomaterial can support the high temperatures and pressures of machines used to finish animal leather.
Bolt Threads (Emeryville, California, USA)	Mycelial cells are fed sawdust and organic material in a tray while controlling the humidity, temperature and other variables.
MycoWorks (Emeryville, California, USA)	Fungal species of the *Ganoderma lucidum* complex (reishi) are grown as mycelium by controlling temperature, humidity, carbon dioxide levels and other aspects of the fungus’s environment.
Hide Biotech (London, UK)	Isolated proteins from scales, bones and other fish waste are engineered with enzymes, chemicals and dyes to create a leather alternative
Modern Meadow (Nutley, New Jersey, USA)	Plant-based proteins combined with a bio-based polyurethane to create a polymer blend called Bio-Alloy

Bucha Bio (New York)	Bacterial microorganisms such as *Gluconacetobacter xylinus* are fermented to produce nanocellulose, which is formulated with plant-based components into various biocomposite materials
Ecovative (Green Island, New York, USA)	Undisclosed fungus strains are grown as mycelium on long beds by controlling the atmosphere and other aspects of the fungus's environment. The mycelial cells are fed agricultural and forestry by-products.

Table 3 Strategies of leather-like material manufacturing from selected companies (Waltz 2022)

A key consideration is whether the use of a supporting scaffold is required during tissue engineering. An example of a company adopting this technique is VitroLabs, where '…immortalized cell lines derived from cells biopsied from an animal grow in a nutrient-rich environment with the help of bio-based scaffolds, forming into tissue with the complexity of an animal hide…' (Waltz 2022). The main processes involved during this method are outlined schematically in Figure7 .

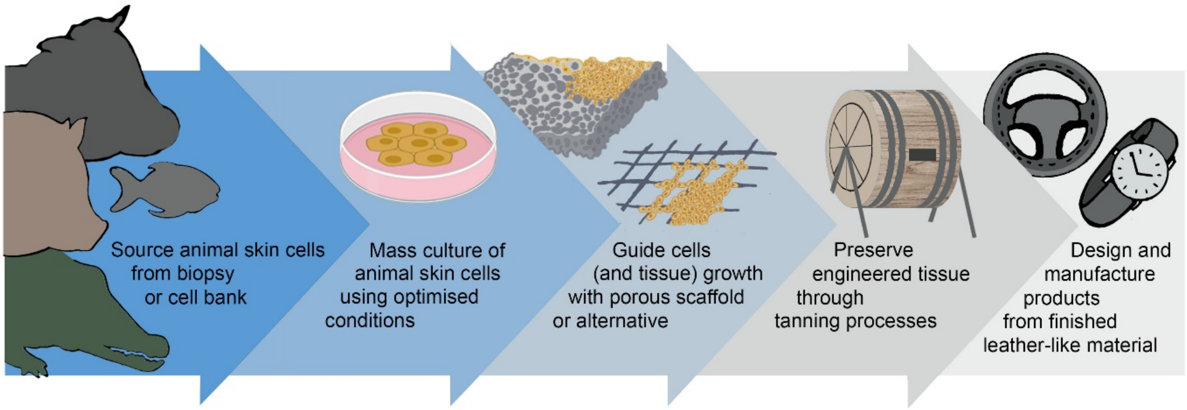

Figure 7: Schematic illustration of the key processes involved in laboratory engineering of a leather-like material using scaffolding technologies. Image created in part with BioRender.com.

5.2.2 Scaffold use

In this section we will highlight latest advantages of using scaffolds and also challenges with future approaches, including their limitations.

Fibrous scaffolds offer the advantage of providing a porous, 3D, mechanical support, that directs nutrient infiltration; cellular growth; and tissue formation (Netti et al., 2005). The structure of the resulting ECM is hence, controlled by the scaffold. The scaffold does not necessarily alter the final material's properties though, as, if biodegradable, the scaffold will not remain in the construct. One option is electrocompaction of collagen into a dense network, using an electric field and subsequent fibrillation (Marga et al., 2017). Dehydration, fibre cross-linking and lubrication can achieve a material with similar properties to tanned leather. Similarly, a non-woven fibrous collagen network may be electrospun in an electric field from a collagen solution (Matthews et al., 2002). Following this, tanning techniques can preserve the protein network, as with animal hides when forming leather (Section 3.3). These strategies may, although laboriously, be scalable through the production of multi-layered constructs. Despite this, a stock source of collagen protein is required. Key drawbacks to this are the expense, impracticality and ethical concerns surrounding extraction of the most commercially available collagen from animal skin (Marga et al., 2017). A research group has been able to recycle collagen waste from leather tanning processes, to generate suitable tissue engineering scaffolds (Netti et al., 2005). This, however, is paradoxical, as production, at least initially, shall rely upon the same industry that a leather substitute would aim to cease. Whilst useful proofs of concept, these methods do not successfully rectify the existing problems with traditional leather manufacture. Sustainable collagen sources should not depend upon animal livestock (Breemhaar and Post, 2019). The use of collagen produced by recombinant plant, bacterial, fungal or mammalian cells could improve the ethics of a final material. Modern Meadow have already successfully genetically engineered recombinant yeast strains to produce collagen for the purpose of leather biofabrication (Dal et al., 2019a, 2019b). Although, this approach would increase the timescale and complexity of overall production methods.

Textile scaffolds are an alternative strategy under investigation, as they are already quick to produce cheaply and on a large scale, with computer-aided design flexibility. Mechanical properties and cellular interactions of the fabrics are highly dependent upon their structure and fibre content (Edwards et al., 2004). Some scaffolds can conveniently provide both firmness and elasticity to the final material, analogous to the properties of real animal skin, as well as support cell and tissue growth during culture. Textile fibres can be spun into yarns, which are then traditionally used to produce woven, knitted or non-woven fabrics. Yarns have superior strength to fibres alone, so can produce more durable scaffolds. Such fabrics are generally on the millimetre thickness scale, which parallels to that desired in the final leather-like material. Modern Meadow present the novel idea that, through a tanning process comparable to during traditional leather manufacture (Section 3.3), a scaffold may be cross-linked to the surrounding tissue expressed by cultured cells (Purcell and Forgacs, 2017). The resultant material is described as a fibre-reinforced composite. Superior durability is possible, compared with degradable scaffolds, which can enable mechanical performance on par with real leather. As the material is not for implantation, biocompatibility is not vital, unlike most medical applications of tissue engineering. For successful cross-linking to collagen protein in tissue though, the scaffold ought to include biological functional groups, such as amine, carboxylic acid, sulfhydryl and hydroxyl. Successful collagen network growth and cross-linking has been achieved with silk protein fibre scaffolds so far. Silk has previously received success within biomaterials generally, due to its biocompatibility, longevity and favourable mechanical properties (Wang et al., 2006). Modern Meadow's material is readily characterised as dissimilar to genuine leather on the microscale, but does achieve the ultimate goal of imitating its look, feel and mechanical performance. It is worth noting however, that silk is a valuable fibre, which increases the cost of an already expensive tissue engineering procedure (Babu, 2015). Silk, could also be a controversial fibre, as it is sourced from silkworms (PETA, 2020b). Target consumers of a laboratory synthesised leather substitute are likely to have concerns about animal product use, so other suitable fibres may need consideration. Silk is also primarily sourced from China, which could add transportation costs and pollution to the life cycle of the final material. Other research groups have suggested that cellulosic and/or synthetic, rather than exclusively protein fibres, could generate suitable scaffold materials and so, provide more sustainable and ethical options (Helgason and Dusko, 2017; Purcell and Forgacs, 2017). With further development, the use of textile scaffolds could, hence, remain a viable strategy for laboratory engineered leather-like materials.

Latest Scaffold Development	Positives and Limitations
Electrocompaction of collagen into a dense network, using an electric field and subsequent fibrillation (Marga et al., 2017)	Key drawbacks to stock source of are the expense, impracticality and ethical concerns surrounding extraction of the most

Non-woven fibrous collagen network electrospun in an electric field from a collagen solution (Matthews et al., 2002).	commercially available collagen from animal skin (Marga et al., 2017). Sustainable collagen sources should not depend upon animal livestock (Breemhaar and Post, 2019)
Modern Meadow present a scaffold that may be cross-linked to the surrounding tissue expressed by cultured cells through a tanning process comparable to traditional leather manufacture (Purcell and Forgacs, 2017)	Superior durability is possible, compared with degradable scaffolds, which can enable mechanical performance on par with real leather. For successful cross-linking to collagen protein in tissue, the scaffold ought to include biological functional groups, such as amine, carboxylic acid, sulfhydryl and hydroxyl.

Table 4 - Latest Developments in Scaffold Use

5.2.3. Advantages of scaffold-free approaches and limitations

In Section 5.2.2 the promise of textile scaffolds was highlighted, yet it is important to note that at present, scaffold free approaches are also being considered. In this section, we will highlight the advantages and limitations of these approaches.

Modern Meadow are developing a leather-like material through scaffold-free tissue engineering technologies. This decision is justified by a scaffold adding unnecessary expense and complication to the manufacturing process (Forgacs et al., 2016). Identification of a compatible biomaterial for a specific cell type is generally a lengthy process of trial and error, that is perhaps appropriate for medicinal purposes, but not for larger scale cellular agriculture (Forgacs et al., 2014). If scaffolds remain in the material, then they can also affect the end structure and properties, so finding an appropriate scaffold can be difficult and require time-consuming testing. 3D scaffolds, of comparable thickness to genuine leather, pose the additional difficulty of achieving complete nutrient and oxygen infiltration, for consistent cell survival throughout. These reservations are valid and Modern Meadow has made impressive progress without needing a scaffold material. On the other hand, the fabrics produced so far do not have equivalent durability to real leather. The material is currently unfit for purpose, as most products require support from an unsustainable plastic-based backing fabric (Jakab et al., 2019). Perhaps in the short term though, this methodology could be a valuable steppingstone, until technology is further refined to allow sufficient durability of cell-based materials in their own right. The lack of fabric strength is likely a result of the current tissue cultivation methodology employed (*Figure 8*). Cells are expanded in 22 x 22 cm^2 culture dishes, then conditions are optimised for collagen secretion. Fresh cells are layered onto the collagen network formed and this process is repeated until three layers fuse together. Triads are then fused to attain a fifteen-layered construct, that undergoes a further three weeks in culture. Scaffolds were not used as ECM mimics to guide cell growth at any point during fabrication. Whilst the technology has some advantages over scaffold utilisation during tissue engineering, the resultant fabric is less robust and thinner than animal skin, being only comparable to the very top grain layer at around 0.1 mm in thickness (*Figure* 3) (Sharphouse, 1995c). Collagen fibres within the engineered sample are fine, also like those found in the grain, with lower entanglement than in real skin (Covington, 2011b). These characteristics prevent the material from performing as well under tensile and tear stress as genuine leather (Kelly et al., 2019). Most leathers contain at least part of the corium layer as well to maintain structural robustness. Thicker, stronger collagen fibres, with greater entanglement, similar to those in the corium, are necessary for generation of a material with comparable mechanical properties to real leather (*Figure* 3) (Dalgado, 2019). The use of a scaffold in tissue engineering can enable greater control over the collagen fibre network structures formed and resultant material properties (Hutmacher, 2001; Jayarama Reddy et al., 2013). Fabrication with a scaffold as a single layer could also improve fibre entanglement. Alternatively, Modern Meadow have suggested that incorporation of chemical binders can increase crosslinking within the collagen network (Jakab et al., 2019). The company may even utilise their past technological developments, regarding cell-based meat, in future engineered leather production. Previously patented is a layer-by-layer bioprinter, which may be able to tackle the issue of finite scaffold thickness, with respect to complete nutrient infiltration and cell survival (Forgacs et al., 2008, 2014). Superior structural control may also be attained through such computer-aided deposition of multi-cellular bodies.

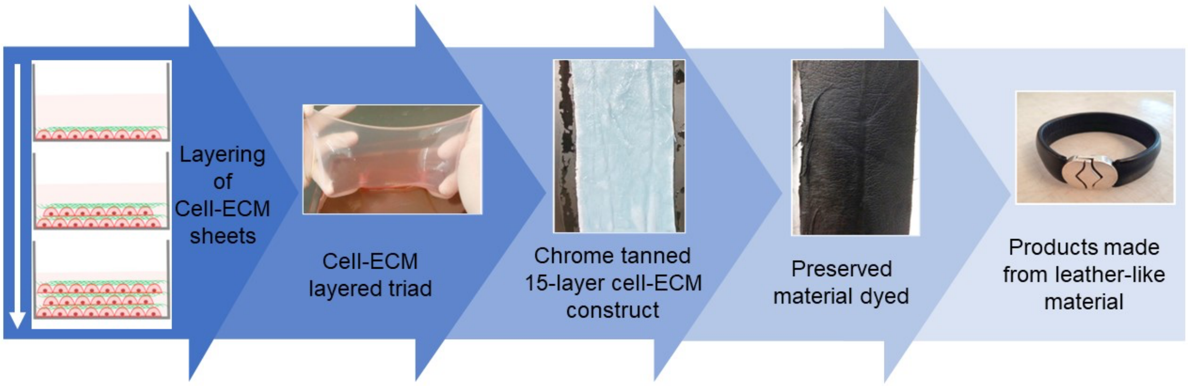

Figure 8: Schematic illustration of leather-like material production in a laboratory using Modern Meadow's scaffold-free technologies. Image adapted from Jakab et al., 2019.

Ultimately, the choice between scaffold and scaffold-free tissue engineering technologies depends upon the properties required by the finished material in its intended purpose. It has, for example, been found that culturing a leather alternative for use in automobile interiors, such as steering wheel covers or seat belt components, is preferable with moulds, rather than scaffolds (Dumbrique et al., 2016). The key advantage is that a leather-like material may be engineered to the final size and shape required, without the need for cutting and sewing of fabric pieces. Waste is minimised, without compromising fabric or product quality. This would be the case with traditional leather from animal hides or, although to a lesser extent, flat pieces of material produced by tissue engineering with a scaffold. The mould method is, on the other hand, currently only suitable for relatively small and thin pieces of leather-like material, that are used primarily for aesthetic purposes. Mechanical support may be introduced by other materials as a backing, if necessary, but this can present sustainability concerns (Jakab et al., 2019). Modern Meadow demonstrates the possibility of decorating textile fabrics with 3D patterns and textures of biofabricated leather material (Lee et al., 2019). The application of a liquid solution of collagen protein to a fabric substrate enables great design flexibility. Again though, sustainability of the technique depends upon collagen sourcing. Until advancement of tissue culture technologies with, for example, cell aggregates (Forgacs et al., 2008), engineering with a scaffold likely remains the most promising option for the production of larger leather-based products that require greater durability, such as jackets and furniture. This suggestion is corroborated by clean meat products, which are currently at a larger scale in development than *in vitro* leather products. These mostly rely upon existing scaffolding technologies, at least in part (Vein, 2004; Van-Eelen et al., 2006; Marga et al., 2015; Elfenbein and Kolbeck, 2018; Ben-Arye and Levenberg, 2019). Although it is worth noting that, so far, most research efforts in laboratory engineered meat have focused upon the refinement of cell culture methods for scale up, before detailed consideration of scaffolds (Genovese et al., 2016; Breemhaar and Post, 2019; Forgacs and Gupta, 2019).

5.3 Overview of Potential Advantages

In this section, we will provide an overview of potential advantages of laboratory engineered leather substitutes. Should technological advances allow laboratory cultivation of a scalable leather substitute in the future, it may more closely imitate the desirable tactile, mechanical and aesthetic properties of genuine leather than existing alternatives (Qu et al., 2008; Marga et al., 2017). Product applications will also be discussed, as well as comparisons with genuine leather, focusing on resources inputs and outputs.

5.3.1 Product applications

In this section, current and future product applications for laboratory engineered leather will be discussed.

Already, several brands, including Nike, have acknowledged many potential design benefits that could be offered over real leather (Greene, 2016). Such a material may be engineered to have specific properties, encompassing texture, shape, size and mechanical performance. Possibilities could range beyond the present restrictions of animal hides. For example, hides are non-uniform, plus inconsistent in quality and size, due to high dependence upon the natural state of reared animals. Engineering under controlled laboratory conditions may enable synthesis of larger pieces, or the exact amount, of material needed, at more consistent quality. Fewer additional processing steps are likely to be required, in comparison to traditional leather manufacture, prior to material preservation during tanning (*Figure* 1). This could be advantageous in terms of waste reduction. Laboratory synthesis also has the potential to engineer any type of animal skin, through structural control of the collagen network formed. Collagen fibre and overall leather thickness, plus resultant physical properties, may be pre-determined during laboratory engineering by, for example, the cell type used (Purcell and Forgacs, 2017). Applicable cell types could go beyond the current range of animal hides used in leather production. For instance, skins that are now banned, or rare, in the leather industry, such as endangered reptile, might be constructed in a laboratory to produce similarly prestigious exotic fabrics (Qiang and Han, 2018). Novel mammal, bird, reptile, fish, amphibian and invertebrate cells, or combinations thereof, may eventually be applied to *in vitro* leather synthesis (Greene, 2016; Helgason and Dusko, 2017; Purcell and Forgacs, 2017). Overall, designers could have a broader range of material options available to them, with simultaneous poaching reduction. This aspect would be popular with fashion leather consumers, who constantly desire new trends (Belleau et al., 2002; Alia et al., 2017).

A key benefit to laboratory engineering of leather substitute materials is that manufacturers should have a lesser dependence upon animal exploitation. Concerns over animal welfare amongst ethically conscious consumers may be tackled, as animal dermal cells can be sourced via harmless biopsies or immortalised cell banks (Purcell and Forgacs, 2017; Jakab et al., 2019). In addition, environmental and sustainability issues concerning animal agriculture could be minimised, as fewer animals are likely to need rearing within the material's life cycle. This aspect and further related advantages are discussed in Section 7. Notably, animal tissue produced within a sterile environment reduces the risk of disease transmission and lowers antibiotic dependence (Birbir et al., 2019; Bhat et al., 2015). As temporary preservation of hides during pre-tanning processes after slaughter will no longer be necessary, the need for antibiotics to prevent putrefaction shall also be removed (Stockman et al., 2007). Availability of active antibiotics is limited, so less reliance during laboratory engineering of leather-like materials would retain these for human treatment and prevent the development of resistant bacterial strains.

5.4 Life Cycle of Laboratory Engineered Leather in Comparison with Genuine Leather

In this section, for comparison with that of real leather (Section 2.1.5., *Figure* 4), the predicted life cycle of a laboratory synthesised leather-like material will be represented in a schematic. All stages, plus the relative inputs and outputs during production, are only estimations for now, as the process is not yet optimised for or performed on a comparable scale to genuine leather manufacture.

5.4.1 Inputs and Outputs

For comparison with that of real leather (Section 2.1.5, *Figure* 4), the predicted life cycle of a laboratory synthesised leather-like material is represented schematically in *Figure* 9. All stages, plus the relative inputs and outputs during production, are only estimations for now, as the process is not yet optimised for or performed on a comparable scale to genuine leather manufacture. It is assumed that the final material shall be a suitable substitute to real leather, for use in equivalent products. The initial stage of production involves engineering structural components of animal skin tissue in a laboratory, in contrast to whole animal agriculture for real leather. The tissue is cultivated from cells, nutrients, growth factors and sterilised matrices (Section 4). Cultured leather should reduce dependence on livestock, which shall in turn lower feed crop, fertiliser, pesticide and land usage (Elfenbein and Kolbeck, 2018). Although, the cells and growth factors used during tissue culture are generally still derived from animals, so a contribution from animal agriculture to the full life cycle cannot yet be entirely eliminated. It currently takes months to rear livestock to a stage where they are large enough to supply suitable hides for the leather industry. Once fully developed, laboratory synthesis could reduce the time needed to produce the same amount of material. At present, a tanned leather-like material may be produced in a laboratory within just six weeks, compared to the months required for genuine leather (Jakab et al., 2019). The exponential growth rate of cells can also provide a more sustainable source of collagenous material, with higher yield (Netti et al., 2005; Arshad et al., 2017). Cellular agriculture is more likely, than animal agriculture, to be capable of keeping up with the future increases in demand for leather-like materials (Dal et al., 2019a, 2019b). A single animal can, however, provide a wide variety of materials to humans, such as milk, meat, fertiliser and medicines, rather than leather alone (Tuomisto, 2019). To fully assess whether cellular agriculture will genuinely reduce environmental impacts compared with animal agriculture, laboratory synthesis of all animal products ought to be considered in the future (Eibl et al., 2018; Stephens and Ellis, 2020).

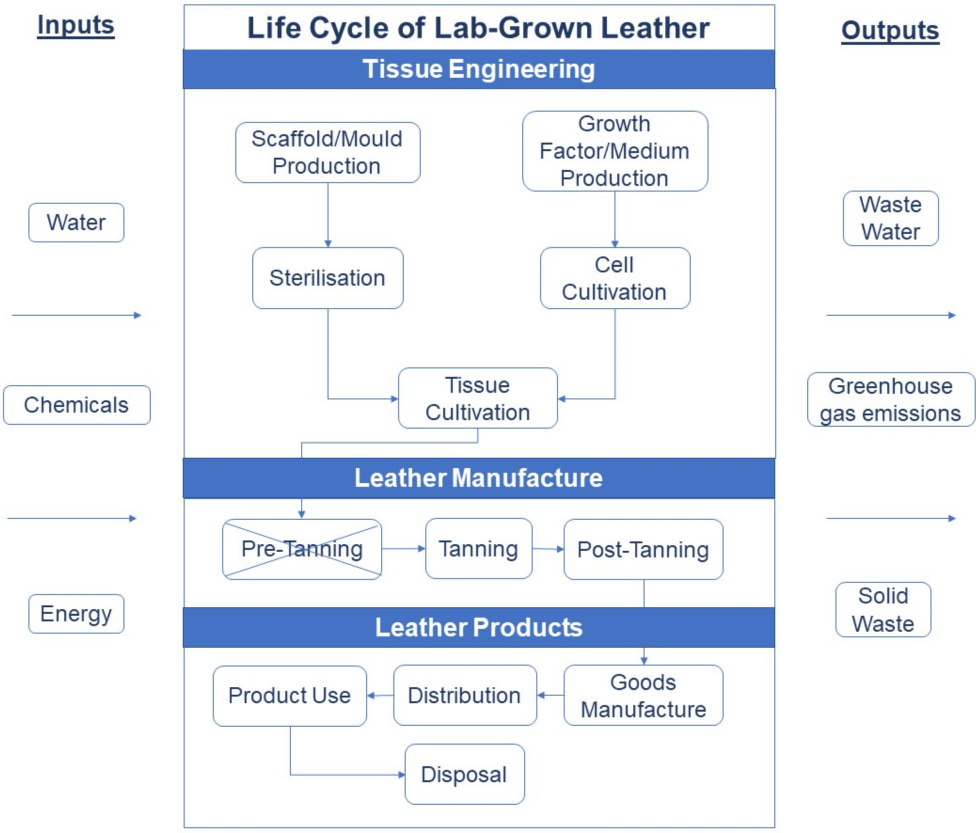

Figure 9: Diagrammatic representation of the predicted life cycle for an in vitro engineered leather substitute, including resource inputs and waste outputs.

Whole animals are comprised of complex biological tissues. Meanwhile, leather itself has the majority of animal skin components removed (Jakab et al., 2019). Most of these then go to waste. Laboratory engineering could eliminate blood vessels and other non-essential components from the offset. Liming procedures, plus consequential toxic pollutants, from traditional leather manufacture (Section 2.1.2 and Table 1), could therefore be avoidable.

In fact, all of the usual pre-tanning stages could be eliminated (*Figure* 1) and production allowed to continue directly from tissue engineering to tanning. Chemical/water input and pollution, plus solid waste output in particular, shall be lowered (Sorolla et al., 2010). The omission of temporary hide preservation, and its associated environmental impacts, is possible through laboratory synthesis, because tissue engineering and tanning can occur sequentially at the same location. Tanning is the major process from traditional leather manufacture that shall need to be carried forward into laboratory synthesis for long-term material preservation (Section 2.1.3.). The combination of tanning agents chosen will depend upon the properties specified within the final leather product. Procedures less harmful to health and the environment should take preference for impact reduction. Most post-tanning steps of production shall still go ahead, in order to achieve the desired final properties (*Figure* 1). Although, generation solely of the necessary collagen network for the final material could eliminate hide splitting into different grades, reducing waste.

In terms of energy usage, laboratory synthesis could significantly lower consumption during transportation especially, as most stages of manufacture are likely to occur in the same location (Jakab et al., 2019). In combination with a rise in vegetarian lifestyles, laboratory engineering should lower farmland and energy use too (Wilson, 1941a). It is, however, worth noting that the relative environmental impacts of *in vitro* and *in vivo* leather production cannot yet be accurately determined, as they do not presently function on equivalent scales. Whilst there shall be a reduction in energy inputs and greenhouse gas emissions owing to animal agriculture, it is likely that the powering of laboratory machinery will be similarly energy intensive. The relative environmental impacts of each manufacturing method will depend upon what sources of energy are used in the future (Tuomisto and Teixeira De Mattos, 2011). Environmental benefits of *in vitro* synthesis relating to climate change may also only be realised if the land where livestock were previously reared is put to efficient use, such as through rewilding (Tuomisto, 2019).

Laboratory cultivated leather-like materials preferably ought to remain biodegradable at the end of their life cycle. This ability may depend upon what matrix materials are utilised during tissue engineering methodology. Some polymeric scaffolds, for example, may be unable to degrade and concurrently increase reliance upon unsustainable fossil fuels. In addition, similarly to genuine leather (Section 2.1.5), biodegradability of the material shall depend upon which chemicals are applied during processing. Irrespective of biodegradability, the material is still likely to offer a more sustainable alternative to leather than the mainly plastic-based synthetics available on the market at present (Dumbrique et al., 2016; Greene, 2016). Unlike polymeric imitation leathers, *in vitro* leather substitutes are expected to have equivalent mechanical properties to real leather and so, shall not compromise durability or quality (Forgacs et al., 2016). Waste generation should, therefore, be minimised, as products that wear out more gradually over time tend to require less regular replacement. Whilst the life cycle inputs and outputs of a laboratory synthesised leather substitute are currently only predictions, there is certainly advantageous potential for an overall reduction in resource consumption and environmental harm. Although, this is under the assumption that ample investment and time shall be allocated to the research area in the future for sufficient technological development and scale up.

6. Possible Limitations

In this section, we will highlight the limitations of laboratory engineered leather substitutes, focusing on the performance and aesthetic properties, production costs and ethical concerns. To do so, we will discuss current limitations, along with the impact of future approaches on the industry.

For example, despite the possible environmental, ethical and health benefits of laboratory engineered leather substitutes (Sections 5.2 and 5.3), there may be some opposition with regards to initial social disadvantages, such as job losses in the farming industry. Nevertheless, the research area remains in its early stages. Scale up of current tissue engineering procedures to meet the demand for a suitable leather alternative is not yet practical or economically viable. It remains unclear whether scaffolding, or other technologies, are the most appropriate strategy, as each method includes drawbacks (Section 5.1).

6.1 Performance and aesthetic properties

Neither approach has, so far, been able to engineer a material with comparable structural complexity or properties to genuine leather. Aside from poor mechanical performance, another drawback of present innovations is that they exclusively appeal to high-end markets (Kansara, 2017). Even the main competitor within the field has not yet been able to commercialise a leather-like material (Modern Meadow, 2020). Products designed for their pilot brand Zoa have remained limited edition samples only. There are several factors that could be responsible for the delay in market entry. Perhaps further publicisation is needed before designers and consumers will accept such a material and enable profitability. Conversely, a higher demand may be expected and hence, a lag time is required to produce initial stocks. Most likely though, the manufacture processes are simply not yet viable or cost-effective in larger quantities.

6.2 Production costs

Currently, a key limitation is that the tissue engineering technologies derived from biomedical applications remain very costly (Bhat and Bhat, 2011; Specht et al., 2018). It is not easy to contemplate how these techniques could directly compete with established leather manufacturing practices on a comparable scale. Significant developments are still needed to achieve a readily scalable fabrication technique.

It is therefore, too soon to generate an accurate cost estimation for laboratory engineered leather-like materials upon market entry. Consumers shall only choose to buy an alternative to real leather if it is of comparable, or greater, quality and similar price to the traditional material. By means of a suitable parallel, the first *in vitro* meat product, released in 2013 merely as a proof of concept, cost at least 300,000 times the price of a classic hamburger (Fountain, 2013). Less than ten years on, companies claim that they can now reduce the cost to around $10 per burger (Gonzalez and Koltrowitz, 2019). Eventually, it is hoped that the product could even become cheaper than traditional meat. Similarly, the expense of laboratory engineered leather-like materials is anticipated to decrease over time, as technical aspects and scale up methods are refined. Leather does however, have the advantage of already being considered a luxury item. High-end fashion products can currently sell for thousands of dollars. Therefore, cost may be less of a limitation for *in vitro* leather and products could actually become competitive within the market sooner than cultured meat.

6.3 Ethical concerns surrounding cell use and sourcing

At present, a distinct limitation of most investigations into laboratory engineered leather-like materials is that successful animal cell proliferation during routine culture relies upon media supplementation with growth promotors, like foetal bovine serum (FBS). There are serious ethical concerns surrounding the sourcing of FBS. Cows pregnant at slaughter have their unborn foetuses removed, then FBS is extracted by cardiac puncture, causing significant distress to both mother and foetus (Van Der Valk et al., 2004). Due to batch variation, the exact constitution of FBS is poorly defined, but it contains essential growth factors and hormones for the stimulation of animal cell proliferation (Maurer, 1986). To remove issues regarding animal cruelty entirely, a replacement for FBS should be considered in the future (Tuomisto, 2019). This could improve marketability of a biofabricated leather-like material amongst increasingly ethically conscious consumers. Growth factor supplements are also the most expensive part of cell culture medium. They are therefore, primarily responsible for the cost and market restrictions of a cultured leather alternative (Tuomisto, 2019). Cell line specific, serum-free medium formulations, or plant-based serums, are potential options for the future (Gstraunthaler, 2003). Although, these require extensive development and tend to be less efficient cell proliferation promoters. Modern Meadow are already considering an alternative solution, whereby collagen is sourced from a non-animal source, such as fungal, bacterial or plant cells (Section 3.2). These methods do however, add the complexity and controversy of genetic engineering to manufacture and they may not be able to produce collagen fibres of comparable strength to those in animal hides. Genetic manipulation of cells is strictly controlled in most countries and some consumers are wary of such practices, so companies remain cautious when proceeding with this approach due to consumer perceptions of genetic modification (Wunderlich, S. et al., 2015). The cultured meat industry, currently more advanced than that of *in vitro* leather, has acknowledged the problems associated with FBS use, along with other common animal derived products (e.g. trypsin), during routine cell culture. Work has already commenced into finding alternatives (Benjaminson et al., 2002; Genovese et al., 2016; Elfenbein and Kolbeck, 2018; Allan et al., 2019; Breemhaar and Post, 2019). Applicable technology may hence have improved sufficiently before a suitable laboratory engineered leather substitute is even identified.

7. Conclusions and Recommendations

In this final section of the paper, conclusions are drawn together to support recommendations for future work. This includes conclusions on the advantages of leather substitutes against traditional approaches, challenges associated with leather substitutes such as costings and consumer acceptance.

7.1. Advantages of leather substitutes vs traditional approaches

A laboratory engineered leather substitute has distinct commercial potential, as demand amongst consumers for more sustainable and ethical materials is increasing (Petter, 2019; Pithers, 2019). The material could offer many benefits compared with genuine leather, including better control over and a greater range of fabric properties. There should also be a lesser dependence upon animal exploitation during production and hence, fewer harmful consequences. Environmental damage may be lowered through reductions in resource use and waste generation. It is likely that there shall be fewer risks to health during manufacture too. These advantages may however, only be realised if technology is capable of advancing sufficiently in the future. All groups presently working in the field have acknowledged shortcomings in their research so far and have begun to explore possible solutions. Examples of key issues that ought to be tackled include the continued reliance upon animal products during routine cell culture and inferior mechanical performance of cultivated materials compared to genuine leather. Research into the topic generally remains in its early phases though, so as this grows, the possibility will hopefully become more achievable, much like the progress being seen towards laboratory cultured meat (Jaso, 2019; Marr, 2019).

7.2. Challenges associated with leather substitutes

7.2.1. Costings

Beyond fine technological developments, one of the next key research steps will focus upon improving cost-efficiency (Jakab et al., 2019). So far, the tissue engineering methods translated from medical applications remain too labour intensive and expensive to replicate the colossal scale of genuine leather production (Breemhaar and Post, 2019). As for all forms of cellular agriculture, automated and scalable bioproduction methods are required before product prices may become more reasonable. Nutritious medium is currently the costliest component of cellular culture. Serum-free formulations remain useful avenues of research, but systems that enable medium recycling would be more beneficial for future cost reduction. Trials have already commenced in relation to cell-based meat, which may later be tailored to other cellular agriculture systems, like leather (Forgacs and Gupta, 2019). In addition, it is possible that genetic manipulation to, for example, generate self-renewing cells, may, regulation permitting, continue to aid cost reduction (Elfenbein and Kolbeck, 2018). Eventually, large scale bioreactors shall ultimately be necessary to meet the high demand for leather-like materials (Allan et al., 2019; Langelaan et al., 2010; Bhat and Fayaz, 2011;). Generally, biological cells are adherent, so rely upon attachment to a substrate, such as a scaffold material, for successful growth and proliferation. Scaffolds are the other major addition to the cost and complexity of classical tissue engineering techniques. They can also restrict material thickness, due to limitations upon cell, oxygen and nutrient infiltration. Alternative methods to control cell growth and the resultant tissue structure will remain important research focusses. Bioprinting techniques could perhaps enhance structural precision during tissue culture (Forgacs et al., 2008). Suspension culture of cells within bioreactors is likely to be more useful when scaling up, so microcarriers, for example, or specialised apparatus, may be utilised in the future (Marga et al., 2015; Breemhaar and Post, 2019). Vital research within the field may however, only continue if further funding is provided (Bhat et al., 2015). As a relatively niche research area to date, it is hoped that this review and future publications shall increase awareness about the concerns over genuine leather and improve understanding of *in vitro* alternatives.

7..2.2. Consumer acceptance of alternatives vs current substitutes vs traditional approach.

Even if technology does advance sufficiently in the future to allow scalable production of cultured leather-like materials, it is as yet uncertain how accepting consumers shall be of such animal product alternatives. Research already indicates apprehension towards cell-based meat and the terms used during advertisement are inconsistent (Siegrist et al., 2018). Lessons shall need to be learnt from this industry as it develops and *in vitro* leather branded according to new market research. Consumers may distrust a new product if reference is made to, for instance, cellular culture or genetic modification. As the foremost company in the field, Modern Meadow actually do not aim to directly compete with the leather industry and instead provide consumers with the option of a novel material (Jakab et al., 2019). It may be that leather itself shall be dissociated from any commercial name for future laboratory engineered alternatives, following a similar marketing trend to plant-based imitation leathers. Consumer perceptions towards these materials shall clearly form another important focus of future research. Overall, it is hoped that the compilation of existing literature within this investigation has increased knowledge in the field and also highlighted gaps in previous research to prompt further work. With this, laboratory engineered alternatives may soon become as popular as genuine leather is today.

Disclosure statement

No potential conflict of interest was reported by the authors.

Acknowledgements

Funding: This work was supported by the Engineering and Physical Sciences Research Council Doctoral Training Award, United Kingdom.

Orcid

Olga Tsigkou - 0000-0002-5250-8610

Celina Jones - 0000-0002-1824-7234

Lucy Bosworth - 0000-0002-6726-4663

References

Abebayehu, T., Kibrom, M., 2010. Study on ectoparasitic defects of processed skins at Sheba Tannery, Tigray, Northern Ethiopia. Trop. Anim. Health Prod. 42, 1719–1722.

Agustini, C.B., Haubert, G., Barcellos, B.S.D.C., Gutterres, M., 2016. Mass reduction of tannery solid wastes by biodegradation. J. Soc. Leather Technol. Chem. 100, 271–274.

Agustini, C.B., Neto, W.L., Priebe, G., Costa, M., Gutterres, M., 2017. Biodegradation of leather solid waste and manipulation of methanogens and chromium-resistant microorganisms. J. Am. Leather Chem. Assoc. 112, 7–14.

Akter, F., Ibanez, J., Bulstrode, N., 2016. Skin Engineering, in: Tissue Engineering Made Easy. Academic Press, London, pp. 17–27. https://doi.org/10.1016/B978-0-12-805361-4.00003-5

Alexander, K.T.W., 1988. Enzymes in the tannery - catalysts for progress? J. Am. Leather Chem. Assoc. 83, 287–316.

Alia, J.P., Ramanathan, G., Fathima, N.N., Uma, T.S., Rao, J.R., 2017. Fish skin and exotic leathers. J. Am. Leather Chem. Assoc. 112, 36–43.

Allan, S.J., De Bank, P.A., Ellis, M.J., 2019. Bioprocess Design Considerations for Cultured Meat

Production With a Focus on the Expansion Bioreactor. Front. Sustain. Food Syst. 3, 44. https://doi.org/10.3389/fsufs.2019.00044

Ammenn, J., Huebsch, C., Schilling, E., Dannheim, B., 2015. Chemistry of syntans and their influence on leather quality. J. Am. Leather Chem. Assoc. 110, 349–354.

Aravindhan, R., Saravanabhavan, S., Rao, J.R., Nair, B.U., Thanikaivelan, P., Chandrasekaran, B., 2004. A Bio-Driven Lime and Pickle Free Tanning Paves Way for Greener Garment Leather Production. J. Am. Leather Chem. Assoc. 99, 53–66.

Ariram, N. and Madhan, B., 2020. Development of bio-acceptable leather using bagasse. Journal of Cleaner Production, 250, p.119441.

Arshad, M.S., Javed, M., Sohaib, M., Saeed, F., Imran, A., Amjad, Z., 2017. Tissue engineering approaches to develop cultured meat from cells: A mini review. Cogent Food Agric. 3, 1320814. https://doi.org/10.1080/23311932.2017.1320814

Avar, G., Meier-Westhues, U., Casselmann, H., Achten, D., 2012. Polyurethanes, in: Polymer Science: A Comprehensive Reference, 10 Volume Set. Elsevier Ltd, Amsterdam, pp. 411–441. https://doi.org/10.1016/B978-0-444-53349-4.00275-2

Azzouz, S., Benmakhlouf, N., Toumi, A., Khedhira, H., Elcafsi, A., 2017. Study of the mechanical characteristics of dried leather. J. Soc. Leather Technol. Chem. 101, 253–257.

Babu, K.M., 2015. Natural Textile Fibres: Animal and Silk Fibres, in: Sinclair, R. (Ed.), Textiles and Fashion: Materials, Design and Technology. Woodhead Publishing Series in Textiles, Cambridge, pp. 57–78.

Bacardit, A., Baquero, G., Sorolla, S., Ollé, L., 2015. Evaluation of a new sustainable continuous system for processing bovine leather. J. Clean. Prod. 101, 197–204. https://doi.org/10.1016/j.jclepro.2015.04.012

Bailey, A.J., Paul, R.G., 1998. Collagen: A not so simple protein. J. Soc. Leather Technol. Chem. 82, 104–110.

Balachandar, V., Arun, M., Mohana Devi, S., Velmurugan, P., Manikantan, P., Karthick Kumar, A., Sasikala, K., Venkatesan, C., 2010. Evaluation of the genetic alterations in direct and indirect exposures of hexavalent chromium [Cr(VI)] in leather tanning industry workers North Arcot District, South India. Int. Arch. Occup. Environ. Health 83, 791–801. https://doi.org/10.1007/s00420-010-0562-y

Bartlett, R., James, B., 1979. Behavior of Chromium in Soils: III. Oxidation. J. Environ. Qual. 8, 31–35. https://doi.org/10.2134/jeq1979.00472425000800010008x

Basak, S., Shakyawar, D.B., Samanta, K.K., Debnath, S., Bhowmick, M. and Kumar, N., 2022. Development of natural fibre based flexural composite: A sustainable mimic of natural leather. Materials Today Communications, 32, p.103976.

Bear, R.S., 1944. Electron microscope images of collagen. J. Am. Chem. Soc. 66, 1297–1305.

Belay, M., Karthikeyan, R., John Sundar, V., Aravindhan, R., 2019. Studies on the Ethiopian camel hides for their suitability for making leather. J. Am. Leather Chem. Assoc. 114, 48–54.

Belleau, B.D., Summers, T.A., Von Hoven, T., 2002. Marketing exotic leather: American alligator, ostrich, and emu. J. Am. Leather Chem. Assoc. 97, 65–73.

Ben-Arye, T., Levenberg, S., 2019. Cultured Meat Compositions. WO 2019/016795 A1.

Benjaminson, M.A., Gilchriest, J.A., Lorenz, M., 2002. In vitro edible muscle protein production system (MPPS): Stage 1, fish. Acta Astronaut. 51, 879–889. https://doi.org/10.1016/S0094-5765(02)00033-4

Bhargavi, N.R.G., Jayakumar, G.C., Sreeram, K.J., Rao, J.R., Nair, B.U., 2015. Towards sustainable leather production: Vegetable tanning in non-aqueous medium. J. Am. Leather Chem. Assoc. 110, 97–102.

Bhat, Z.F., Bhat, H., 2011. Animal-free meat biofabrication. Am. J. Food Technol. 6, 441–459. https://doi.org/10.3923/ajft.2011.441.459

Bhat, Z.F., Fayaz, H., 2011. Prospectus of cultured meat - Advancing meat alternatives. J. Food Sci. Technol. 48, 125–140. https://doi.org/10.1007/s13197-010-0198-7

Bhat, Z.F., Kumar, S., Fayaz, H., 2015. In vitro meat production: Challenges and benefits over conventional meat production. J. Integr. Agric. 14, 241–248. https://doi.org/10.1016/S2095-3119(14)60887-X

Bielak, E. and Marcinkowska, E., 2022. Heavy metals in leathers, artificial leathers, and textiles in the context of quality and safety of use. Scientific Reports, 12(1), p.5061.

Birbir, M., Yazici, E., Caglayan, P., 2019. Investigation of antibiotic-resistant Enterobacteriaceae isolated from soaked sheepskins and cattle hides. J. Soc. Leather Technol. Chem. 103, 6–13.

Birnbaum, D.P., Manjula-Basavanna, A., Kan, A., Tardy, B.L. and Joshi, N.S., 2021. Hybrid living capsules autonomously produced by engineered bacteria. Advanced Science, 8(11), p.2004699.

Bitlislp, B.O., Karavana, H.A., Basaran, B., Aslan, A., 2005. Importance of using genuine leather in shoe production in terms of foot comfort. J. Soc. Leather Technol. Chem. 89, 107–110.

Bolt Threads, 2020. Meet Mylo [WWW Document]. URL https://boltthreads.com/technology/mylo/ (accessed 3.20.20).

Bosnic, M., Buljan, J., Daniels, R.P., 2000. Pollutants in tannery effluents, United Nations Industrial Development Organization. Vienna.

Breemhaar, J.J., Post, M., 2019. Apparatus and Process for Production of Tissue from Cells. US 2019/0338232 A1.

Brindha, K., Elango, L., 2012. Impact of Tanning Industries on Groundwater Quality near a Metropolitan City in India. Water Resour. Manag. 26, 1747–1761. https://doi.org/10.1007/s11269-012-9985-4

Brown, E.M., Taylor, M.M., Marmer, W.N., 1996. Production and potential uses of co-products from solid tannery waste. J. Am. Leather Chem. Assoc. 91, 270–276.

Brugnoli, F., 2017. Sustainability in the leather value chain: Global overview, regional and sectoral peculiarities. J. Am. Leather Chem. Assoc. 112, 286–293.

Butler, L.G., Head, G.M., 1993. The medium frequency (7.5 MHz) ultrasound image characteristics of cattle skin. Aust. Vet. J. 70, 344–347. https://doi.org/10.1111/j.1751-0813.1993.tb00881.x

Camargo, J.A., Alonso, Á., 2006. Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: A global assessment. Environ. Int. 32, 831–849. https://doi.org/10.1016/j.envint.2006.05.002

Carrara, J., 2018. Fashion: Fabrics of the Future [WWW Document]. ECO-AGE. URL https://eco-age.com/news/fabrics-future (accessed 3.20.20).

Chattopadhyay, B., Aich, A., Mukhopadhyay, S.K., 2012. Chromium in the tanning industry: An odyssey from cradle to grave. J. Soc. Leather Technol. Chem. 96, 133–140.

Chen, Mianhong, Duan, Y., Dong, L., Chen, Min, Cheng, H., 2019. Country-level Life cycle assessment of carbon footprint in processing of bovine upper leather. J. Am. Leather Chem. Assoc. 114, 194–203.

China, C.R., Hilonga, A., Nyandoro, S.S., Schroepfer, M., Kanth, S.V., Meyer, M. and Njau, K.N., 2020. Suitability of selected vegetable tannins traditionally used in leather making in Tanzania. Journal of Cleaner Production, 251, p.119687.

Chouhan, D., Dey, N., Bhardwaj, N., Mandal, B.B., 2019. Emerging and innovative approaches for wound healing and skin regeneration: Current status and advances. Biomaterials. https://doi.org/10.1016/j.biomaterials.2019.119267

Chowdhury, Z.U.M., Ahmed, T., Antunes, A.P.M., Paul, H.L., 2018. Environmental life cycle assessment of leather processing industry: A case study of Bangladesh. J. Soc. Leather Technol. Chem. 102, 18–26.

Chriki, S., Hocquette, J.-F., 2020. The Myth of Cultured Meat: A Review. Front. Nutr. 7, 1–9. https://doi.org/10.3389/fnut.2020.00007

Çolak, S., Zengin, G., Özgünay, H., Sari, Ö., Sarikahya, H., Yüceer, L., 2005. Utilization of leather industry pre-fleshings in biodiesel production. J. Am. Leather Chem. Assoc. 100, 137–141.

Cooman, K., Gajardo, M., Nieto, J., Bornhardt, C., Vidal, G., 2003. Tannery wastewater characterization and toxicity effects on Daphnia spp. Environ. Toxicol. 18, 45–51. https://doi.org/10.1002/tox.10094

Covington, A.D., 2011a. Introduction, in: Tanning Chemistry - The Science of Leather. RSC Publishing, Cambridge, pp. xix–xxiii.

Covington, A.D., 2011b. Skin and its Components, in: Tanning Chemistry - The Science of Leather. RSC Publishing, Cambridge, pp. 29–69.

Covington, A.D., 2011c. Collagen and Skin Structure, in: Tanning Chemistry - The Science of Leather. RSC Publishing, Cambridge, pp. 1–17.

Covington, A.D., 2011d. Liming, in: Tanning Chemistry - The Science of Leather. RSC Publishing, Cambridge, pp. 134–153.

Covington, A.D., 2011e. Tanning, in: Tanning Chemistry - The Science of Leather. RSC Publishing, Cambridge, pp. 195–203.

Covington, A.D., 2011f. Mineral Tanning: Chromium (III), in: Tanning Chemistry - The Science of Leather. RSC Publishing, Cambridge, pp. 204–258.

Covington, A.D., 2011g. Other Tannages, in: Tanning Chemistry - The Science of Leather. RSC Publishing, Cambridge, pp. 315–347.

Covington, A.D., 2011h. Dyeing, in: Tanning Chemistry - The Science of Leather. RSC Publishing, Cambridge, pp. 370–391.

Covington, A.D., 2011i. Fatliquoring, in: Tanning Chemistry - The Science of Leather. RSC Publishing, Cambridge, pp. 392–420.

Covington, A.D., 1986. The Use of Aluminium(III) to Improve Chrome Tannage. J. Soc. Leather Technol. Chem. 70, 33.

Covington, A.D., Sykes, R.L., 1984. The use of aluminium salts in tanning. J. Am. Leather Chem. Assoc. 79, 72–93.

Dal, L., Borden, J., Nelson, J., Ruebling-Jass, K., 2019a. Yeast Strains and Methods for Producing Collagen. US 2019/0002893 A1.

Dal, L., Ruebling-Jass, K., Williamson, D.T., 2019b. Recombinant Yeast Strains. US 2019/0093116 A1.

Dalgado, 2019. All You Need To Know About Leather Grades [WWW Document]. URL https://dalgado.de/en/the-journal/all-about-leather/all-you-need-to-know-about-leather-grades (accessed 5.6.19).

D.A. Littler, A.W. Pearson, 1972. Marketing a new industrial good: A case study, Industrial Marketing Management, Volume 1, Issue 3, Pages 299-307, ISSN 0019-8501. https://doi.org/10.1016/0019-8501(72)90022-3.

De Ponte, C., Liscio, M.C. and Sospiro, P., 2023. State of the art on the Nexus between sustainability, fashion industry and sustainable business model. Sustainable Chemistry and Pharmacy, 32, p.100968.

Dettmer, A., Dos Anjos, P.S., Gutterres, M., 2013. Special review paper: Enzymes in the leather industry. J. Am. Leather Chem. Assoc. 108, 146–158.

Dettmer, A., Coelho Cavalheiro, J., Cavalli, E., Misturini Rossi, D., de Souza Gusatti, C., Zachia Ayub, M.A. and Gutterres, M., 2012. Optimization of the biotechnological process for hide unhairing in substitution of toxic sulfides. Chemical engineering & technology, 35(5), pp.803-810.

Didato, D.T., Steele, S.R., Stockman, G.B., Bailey, D.G., 2008. Recent developments in the short-term preservation of cattle hides. J. Am. Leather Chem. Assoc. 103, 383–392.

Dixit, S., Yadav, A., Dwivedi, P.D., Das, M., 2015. Toxic hazards of leather industry and technologies to combat threat: A review. J. Clean. Prod. 87, 39–49. https://doi.org/10.1016/j.jclepro.2014.10.017

Dowling, D.F., 1955. The thickness of cattle skin. Aust. J. Agric. Res. 6, 776–785. https://doi.org/10.1071/AR9550776

Dumbrique, C.C., Vile, A.T., Siddharthan, C., Hernandez, U.P., 2016. Engineered Leather for Airbag, Steering Wheel and Seatbelt Applications. US 2016/0097154 A1.

Dunhill, K.G., Jacklin, C.N., Grant, S.M., 1990. Environmentally-friendly production of chrome-tanned leathers. J. Am. Leather Chem. Assoc. 85, 225–233.

Edwards, S.L., Mitchell, W., Matthews, J.B., Ingham, E., Russell, S.J., 2004. Design of nonwoven scaffold structures for tissue engineering of the anterior cruciate ligament, in: Autex Research Journal. pp. 86–94.

Eibl, R., Meier, P., Stutz, I., Schildberger, D., Hühn, T., Eibl, D., 2018. Plant cell culture technology in the cosmetics and food industries: current state and future trends. Appl. Microbiol. Biotechnol. 102, 8661–8675. https://doi.org/10.1007/s00253-018-9279-8

Elfenbein, A., Kolbeck, J.L., 2018. Ex Vivo Meat Production. WO 2018/227016 A1.

Fathima, N.N., Aravindhan, R., Rao, J.R., Nair, B.U., 2006. Tannic acid-phosphonium combination: A versatile chrome-free organic tanning. J. Am. Leather Chem. Assoc. 101, 161–168.

Ferreira, M.J., Almeida, M.F., Pinho, S.C., Santos, I.C., 2010. Finished leather waste chromium acid extraction and anaerobic biodegradation of the products. Waste Manag. 30, 1091–1100. https://doi.org/10.1016/j.wasman.2009.12.006

Forgacs, G., Gupta, N., 2019. Large Scale Cell Culture System for Making Meat and Associated Products. US 2019/0376026 A1.

Forgacs, G., Jakab, K., Neagu, A., Mironov, V., 2008. Self-Assembling Cell Aggregates and Methods of Making Engineered Tissue Using the Same. US 2008/0070304 A1.

Forgacs, G., Marga, F., Jakab, K.R., 2014. Engineered Comestible Meat. US 8703216 B2.

Forgacs, G., Marga, F.S., Jakab, K.R., 2016. Engineered Leather and Methods of Manufacture Thereof. US 2016/0097109 A1.

Fountain, H., 2013. A Lab-Grown Burger Gets a Taste Test. New York Times 5.

Gandia, A., van den Brandhof, J.G., Appels, F.V. and Jones, M.P., 2021. Flexible fungal materials: shaping the future. Trends in Biotechnology, 39(12), pp.1321-1331.

Gbolagunte, G.D., Hambolu, J.O., 2010. Types and frequency of isolation of bacteria affecting leather surfaces fromsm all nigerian ruminant dermatoses. J. Soc. Leather Technol. Chem. 94, 102–105.

Genovese, N.J., Roberts, R.M., Telugu, B.P., 2016. Method for Scalable Skeletal Muscle Lineage Specification and Cultivation. US 2016/0251625 A1.

George, N., Chauhan, P.S., Kumar, V., Puri, N. and Gupta, N., 2014. Approach to ecofriendly leather: characterization and application of an alkaline protease for chemical free dehairing of skins and hides at pilot scale. Journal of cleaner production, 79, pp.249-257.

Ghodbane, S.A., Dunn, M.G., 2016. Physical and mechanical properties of cross-linked type I collagen scaffolds derived from bovine, porcine, and ovine tendons. J. Biomed. Mater. Res. - Part A 104, 2685–2692. https://doi.org/10.1002/jbm.a.35813

Givhan, R., 2015. Luxury fashion brands are going green. But why are they keeping it a secret? Washington Post.

Gonzalez, A., Koltrowitz, S., 2019. The $280,000 lab-grown burger could be a more palatable $10 in two years. Reuters.

Grand View Research, 2021 Leather Goods Market Growth & Trends (2021) Available at: https://www.grandviewresearch.com/press-release/global-leather-goods-market (Accessed 13 February 2023)

Grassmann, W., Hormann, H., Hafter, R., 1957. Related to collagen amino acid sequence. Z. Physiol. Chem. 307, 87–96.

Green, M., 1985. Cr Chromium, in: Cardin, D.J. (Ed.), Organometallic Compounds of the Lanthanides, Actinides and Early Transition Metals. Springer, New York, pp. 12–39. https://doi.org/10.1007/978-1-4899-7164-7_6

Greene, P.S., 2016. Footwear and Other Products Incorporating Cultured Leather. US 9428817 B2.

Gross, J., Schmitt, F.O., 1948. The Structure of Human Skin Collagen as Studied with the Electron Microscope. J. Exp. Med. 88, 555–568.

Gstraunthaler, G., 2003. Alternatives to the use of fetal bovine serum: serum-free cell culture. ALTEX 20, 275–81.

Guida, M., Giorgio, A., Aveta, R., Scotti, M., Caracciolo, D., Libralato, G., Aliberti, F., Bonis, S. De, Naviglio, B., 2019. Biodegradability of “eco-friendly” Leather using Respirometric Approach. J. Am. Leather Chem. Assoc. 114, 325–332.

Guo-Tao, F., Zhi-Hua, S., Shuqing, L., Hui, C., 2013. The production of organic fertilizer using tannery sludge. J. Am. Leather Chem. Assoc. 108, 189–196.

Gutterres, M., Passos, J.B., Aquim, P.M., Severo, L.S., Trierweiler, J.O., 2008. Reduction of water demand and treatment cost in tanneries through reuse technique. J. Am. Leather Chem. Assoc. 103, 138–143.

Hadley, P.J., Garnsworthy, P.C., Yanek, S., Shelly, D., 2005. Raw Material Quality Issues. J. Am. Leather Chem. Assoc. 100, 142–148.

Haines, B.M., Barlow, J.R., 1975. The anatomy of leather. J. Mater. Sci. https://doi.org/10.1007/BF00543698

Hall, C.E., Jakus, M.A., Schmitt, F.O., 1942. Electron Microscope Observations of Collagen. J. Am. Chem. Soc. 64, 1234.

Hansen, É., de Aquim, P.M. and Gutterres, M., (2021a). Current technologies for post-tanning wastewater treatment: A review. Journal of Environmental Management, 294, p.113003. (a)

Hansen, É., Cardoso, J.K., Gutterres, M. and de Aquim, P.M., (2021b) Scale-up testing for reducing pollution load of chemicals in wastewater of leather post-tanning. Process Safety and Environmental Protection, 155, pp.466-472. (b)

Hansen, É., de Aquim, P.M., Hansen, A.W., Cardoso, J.K., Ziulkoski, A.L. and Gutterres, M., 2020.

Impact of post-tanning chemicals on the pollution load of tannery wastewater. Journal of Environmental Management, 269, p.110787. (c)

Hao, D., Wang, X., Liang, S., Yue, O., Liu, X., Hao, D. and Dang, X., 2023. Sustainable leather making—An amphoteric organic chrome-free tanning agents based on recycling waste leather. Science of The Total Environment, p.161531.

Haslam, E., 1997. Vegetable tannage: Where do the tannins go? J. Soc. Leather Technol. Chem. 81, 45–51.

He, Q., Yao, K., Sun, D., Shi, B., 2005. Environmental impact evaluation of typical beamhouse processes in leather manufacture based on wastewater settleability. J. Am. Leather Chem. Assoc. 100, 473–480.

Helgason, I., Dusko, I., 2017. Engineered Skin Equivalent, Method of Manufacture Thereof and Products Derived Therefrom. US 2017/0306428 A1.

Hildebrandt, J., Thrän, D. and Bezama, A., 2021. The circularity of potential bio-textile production routes: comparing life cycle impacts of bio-based materials used within the manufacturing of selected leather substitutes. Journal of Cleaner Production, 287, p.125470.

Hodder, J.J., 1995. Waterproof leather technologies and processes. J. Am. Leather Chem. Assoc. 90, 82–87.

Hofman, K., Hall, B., Cleaver, H., Marshall, S., 2011. High-throughput quantification of hydroxyproline for determination of collagen. Anal. Biochem. 417, 289–291. https://doi.org/10.1016/j.ab.2011.06.019

Horch, R.E., Kopp, J., Kneser, U., Beier, J., Bach, A.D., 2005. Tissue engineering of cultured skin substitutes. J. Cell. Mol. Med. 9, 592–608. https://doi.org/10.1111/j.1582-4934.2005.tb00491.x

Howard, G.T., 2002. Biodegradation of polyurethane: A review. Int. Biodeterior. Biodegrad. 49, 245–252. https://doi.org/10.1016/S0964-8305(02)00051-3

Hüffer, S., Taeger, T., 2004. Sustainable leather manufacturing - A topic with growing importance. J. Am. Leather Chem. Assoc. 99, 424–428.

Hutmacher, D.W., 2001. Scaffold design and fabrication technologies for engineering tissues - State of the art and future perspectives. J. Biomater. Sci. Polym. Ed. 12, 107–124. https://doi.org/10.1163/156856201744489

Ilou, I., Souabi, S., Digua, K., 2014. Quantification of Pollution Discharges from Tannery Wastewater and Pollution Reduction by Pre-Treatment Station. Int. J. Sci. Res. 3, 1706–1715.

Jakab, K., Marga, F., Kaesser, R., Chuang, T.-H., Varadaraju, H., Cassingham, D., Lee, S., Forgacs, A., Forgacs, G., 2019. Non-medical applications of tissue engineering: biofabrication of a leather-like material. Mater. Today Sustain. 5, 100018. https://doi.org/10.1016/j.mtsust.2019.100018

Jaso, M., 2019. Plant-based Meat Could Create a Radically Different Food Chain. Econ.

Jayarama Reddy, V., Radhakrishnan, S., Ravichandran, R., Mukherjee, S., Balamurugan, R., Sundarrajan, S., Ramakrishna, S., 2013. Nanofibrous structured biomimetic strategies for skin tissue regeneration. Wound Repair Regen. https://doi.org/10.1111/j.1524-475X.2012.00861.x

Jiang, J., Zhao, J., 2014. Hazards, regulations and alternatives of restricted substances in leather/textile industry: Hexavalent chromium. J. Soc. Leather Technol. Chem. 98, 177–181.

JJ. Tancous, 1969. ISOLATION OF CARBOHYDRATE-CONTAINING COMPONENTS FROM CORIUM OF STEERHIDE. Am Leather Chem. Assn-J 64, 388–403.

Johnson, A., 2020. Agritech Patent Watch [WWW Document]. Explor. Biotech. URL https://explorebiotech.com/patent-watch-lab-grown-leather/ (accessed 4.27.20).

Jones, M., Gandia, A., John, S. and Bismarck, A., 2021. Leather-like material biofabrication using fungi. Nature Sustainability, 4(1), pp.9-16.

Joseph, K., Nithya, N., 2009. Material flows in the life cycle of leather. J. Clean. Prod. 17, 676–682. https://doi.org/10.1016/j.jclepro.2008.11.018

Kanagaraj, G., Elango, L., 2016. Hydrogeochemical processes and impact of tanning industries on groundwater quality in Ambur, Vellore district, Tamil Nadu, India. Environ. Sci. Pollut. Res. 23, 24364–24383. https://doi.org/10.1007/s11356-016-7639-4

Kanagaraj, J., Babu, N.C., Sadulla, S., Rajkumar, G.S., Visalakshi, V. and Kumar, N.C., 2001. Cleaner techniques for the preservation of raw goat skins. Journal of Cleaner Production, 9(3), pp.261-268.

Kanagaraj, J., Panda, R.C. and Kumar, V., 2020. Trends and advancements in sustainable leather processing: Future directions and challenges—A review. Journal of Environmental Chemical Engineering, 8(5), p.104379.

Kanagaraj, J., Panda, R.C. and Senthilvelan, T., 2016. Green remediation of sulfide in oxidative dehairing of skin and correlation by mathematical model: an eco-friendly approach. Process safety and environmental protection, 100, pp.36-48.

Kanagaraj, J., Sastry, T.P. and Rose, C., 2005. Effective preservation of raw goat skins for the reduction of total dissolved solids. Journal of Cleaner Production, 13(9), pp.959-964.

Kanigel, R. (2010). Faux real : genuine leather and 200 years of inspired fakes. Paperback edition. Philadelphia, Pennsylvania: University of Pennsylvania Press.

Kansara, V.A., 2017. With Lab-Grown Leather, Modern Meadow Is Engineering a Fashion Revolution. Bus. Fash.

Karthikeyan, R., Chandra Babu, N.K., 2017. An investigation on chicken leg skin for the preparation of fashionable leather and leather products. J. Am. Leather Chem. Assoc. 112, 190–197.

Karunyal, S., Renuga, G., Kailash, P., 1994. Effects of tannery effluent on seed germination, leaf area, biomass and mineral content of some plants. Bioresour. Technol. 47, 215–218. https://doi.org/10.1016/0960-8524(94)90183-X

Kelly, S.J., Edmonds, R.L., Cooper, S., Sizeland, K.H., Wells, H.C., Ryan, T., Kirby, N., Hawley, A., Mudie, S., Haverkamp, R.G., 2018. Mapping tear strength and collagen fibril orientation in bovine, ovine and cervine hides and skins. J. Am. Leather Chem. Assoc. 113, 1–11.

Kelly, S.J.R., Weinkamer, R., Bertinetti, L., Edmonds, R.L., Sizeland, K.H., Wells, H.C., Fratzl, P., Haverkamp, R.G., 2019. Effect of collagen packing and moisture content on leather stiffness. J. Mech. Behav. Biomed. Mater. 90, 1–10. https://doi.org/10.1016/j.jmbbm.2018.10.004

Kirsner, R.S., Falanga, V., Eaglstein, W.H., 1998. The development of bioengineered skin. Trends Biotechnol. 16, 246–249. https://doi.org/10.1016/S0167-7799(98)01196-2

Kobayashi, A., Takehana, K., Eerdunchaolu, Iwasa, K., Abe, M., Yamaguchi, M., 1999. Morphometric analysis of collagen: A comparative study in cow and pig skins. Anat. Histol. Embryol. 28, 235–238. https://doi.org/10.1046/j.1439-0264.1999.00196.x

Kolomaznik, K., Blaha, A., Dedrle, T., Bailey, D.G., Taylor, M.M., 1996. Non-ammonia deliming of cattle hides with magnesium lactate. J. Am. Leather Chem. Assoc. 91, 18–20.

Konrad, C., Bichler, B., Lorber, K., Marquez, F., 2000. Input/output analysis at a chilean tannery. J. Soc. Leather Technol. Chem. 84, 223–226.

Koppany, J., 2004. A geopolitical essay of the leather industry over the past 50 years. J. Am. Leather Chem. Assoc. 99, 485–493.

Kowalska, M., Zbikowska, A., Wozniak, M., 2019. Innovative Approach to Sustainable Leather Tanning Process using a Lactic Acid Based Agent. J. Am. Leather Chem. Assoc. 115, 443–449.

Langelaan, M.L.P., Boonen, K.J.M., Polak, R.B., Baaijens, F.P.T., Post, M.J., van der Schaft, D.W.J., 2010. Meet the new meat: tissue engineered skeletal muscle. Trends Food Sci. Technol. 21, 59–66. https://doi.org/10.1016/j.tifs.2009.11.001

Laurenti, R., Redwood, M., Puig, R., Frostell, B., 2017. Measuring the Environmental Footprint of Leather Processing Technologies. J. Ind. Ecol. 21, 1180–1187. https://doi.org/10.1111/jiec.12504

Lee, S., Congdon, C.A., Schneider, M., Purcell, B.P., Clayton, C.M., Krasnodebska, N., Muse, N., 2019. Biofabricated Leather Articles, and Methods Thereof. US 2019/0024303.

Legesse, W., Thanikaivelan, P., Rao, J.R., Nair, B.U., 2002. Underlying principles in chrome tanning: Part 1. Conceptual design of pickle-less tanning. J. Am. Leather Chem. Assoc. 97, 475–486.

Li, B., Liao, P., Xie, L., Li, Q., Pan, C., Ning, Z. and Liu, C., 2020. Reduced NOM triggered rapid Cr (VI) reduction and formation of NOM-Cr (III) colloids in anoxic environments. Water Research, 181, p.115923.

Lindner, W., Neuber, H.U., 1990. Preservation in the tannery. Int. Biodeterior. 26, 195–203. https://doi.org/10.1016/0265-3036(90)90059-G

Liu, X., Wang, Y., Wang, X., Han, T., Wang, W. and Jiang, H., 2022. A salt-free pickling and chrome-free tanning technology: a sustainable approach for cleaner leather manufacturing. Green Chemistry, 24(5), pp.2179-2192.

Ma, J., Hou, X., Gao, D., Lv, B. and Zhang, J., 2014. Greener approach to efficient leather soaking process: role of enzymes and their synergistic effect. Journal of cleaner production, 78, pp.226-232.

MacNeil, S., 2008. Biomaterials for tissue engineering of skin. Mater. Today 11, 26–35. https://doi.org/10.1016/S1369-7021(08)70087-7

MacNeil, S., 2007. Progress and opportunities for tissue-engineered skin. Nature 445, 874–880. https://doi.org/10.1038/nature05664

Madhan, B., Aravindhan, R., Siva, M.S., Sadulla, S., Rao, J.R. and Nair, B.U., 2006. Interaction of aluminum and hydrolysable tannin polyphenols: an approach to understanding the mechanism of aluminum vegetable combination tannage. Journal of the American Leather Chemists Association.

Mansour, H.M., Sohn, M.J., Al-Ghananeem, A., DeLuca, P.P., 2010. Materials for pharmaceutical dosage forms: Molecular pharmaceutics and controlled release drug delivery aspects. Int. J. Mol. Sci. 11, 3298–3322. https://doi.org/10.3390/ijms11093298

Marga, F.S., Jakab, K.R., Purcell, B., Williamson, D., 2017. Method for Making Electrocompacted Leather. EP 3 205 754 A1.

Marga, F.S., Purcell, B.P., Forgacs, G., Forgacs, A., 2015. Edible and Animal-Product-Free Microcarriers for Engineered Meat. US 2015/0079238 A1.

Marr, B., 2019. The Future of Food: Amazing Lab Grown and 3D Printed Meat and Fish. Forbes.

Matthews, J.A., Wnek, G.E., Simpson, D.G., Bowlin, G.L., 2002. Electrospinning of collagen nanofibers. Biomacromolecules 3, 232–238. https://doi.org/10.1021/bm015533u

Maurer, H., 1986. Towards chemically-defined, serum-free media for mammalian cell culture, in: Freshney, R. (Ed.), Animal Cell Culture: A Practical Approach. IRL Press, Oxford, pp. 13–32.

Meadow, M., 2020. Our Technology [WWW Document]. URL http://www.modernmeadow.com/our-technology/ (accessed 3.24.20).

Meikle, J.L. (1995). Presenting a New material: From Imitation to Innovation with Fabrikoid. The journal of the Decorative Arts Society 1850--the Present, 19(19), pp.8–15.

Mersiowsky, I., Weller, M., Ejlertsson, J., 2001. Fate of plasticised PVC products under landfill conditions: A laboratory-scale landfill simulation reactor study. Water Res. 35, 3063–3070. https://doi.org/10.1016/S0043-1354(01)00027-6

Modern Meadow [WWW Document], 2020. URL http://www.modernmeadow.com/ (accessed 1.14.20).

Mondal, N.C., Saxena, V.K., Singh, V.S., 2005. Assessment of groundwater pollution due to tannery industries in and around Dindigul, Tamilnadu, India. Environ. Geol. 48, 149–157. https://doi.org/10.1007/s00254-005-1244-z

Moroni, L., De Wijn, J.R., Van Blitterswijk, C.A., 2008. Integrating novel technologies to fabricate smart scaffolds. J. Biomater. Sci. Polym. Ed. 19, 543–572. https://doi.org/10.1163/156856208784089571

Narayanan, K.B., Zo, S.M. and Han, S.S., 2020. Novel biomimetic chitin-glucan polysaccharide nano/microfibrous fungal-scaffolds for tissue engineering applications. International journal of biological macromolecules, 149, pp.724-731.

Naffa, R., Maidment, C., Holmes, G., Norris, G., 2019. Insights into the molecular composition of the skins and hides used in leather manufacture. J. Am. Leather Chem. Assoc. 114, 29–37.

Netti, P.A., Rossi, M., Brugnoli, F., Ioannidis, I., Battista, S., 2005. Tissue of Animal Origin for Industrial Uses Constituted of Material in Sheets, in Vitro Processes for the Production Thereof. EP 1 589 098 A1.

Nichols, B., 2013. Cattle wait to be immunized on a Texas farm: US Department of Agriculture [WWW Document]. Flickr under CC BY 2.0. URL https://www.flickr.com/photos/41284017@N08/9679055249 (accessed 6.30.20).

NSW Government: Department of Primary Industries, 2019. Fertilisers and the environment [WWW Document]. URL https://www.dpi.nsw.gov.au/agriculture/soils/improvement/environment (accessed 6.2.19).

O'Mara, F.P., 2011. The significance of livestock as a contributor to global greenhouse gas emissions today and in the near future. Anim. Feed Sci. Technol. 166–167, 7–15. https://doi.org/10.1016/j.anifeedsci.2011.04.074

Ollé, L., Jorba, M., Font, J., Shendrik, A., Bacardit, A., 2011. Biodegradation of wet-white leather. J. Soc. Leather Technol. Chem. 95, 116–120.

Ortiz-Monsalve, S., Valente, P., Poll, E., Jaramillo-García, V., Henriques, J.A.P. and Gutterres, M., 2019. Biodecolourization and biodetoxification of dye-containing wastewaters from leather dyeing by the native fungal strain Trametes villosa SCS-10. Biochemical Engineering Journal, 141, pp.19-28.

Parry, D., Craig, A., 1988. Collagen Fibrils During Development and Maturation and their Contribution to the Mechanical Attributes of Connective Tissue, in: Collagen. CRC Press, Boca Raton, Florida, p. 2.

Patowary, K., 2014. The Leather Tanneries of Fez, Morocco [WWW Document]. Amusing Planet. URL https://www.amusingplanet.com/2014/08/the-leather-tanneries-of-fez-morocco.html (accessed 6.29.20).

Paul, H., Antunes, A.P., Covington, A.D., Evans, P., Philips, P.S., 2013. Towards zero solid waste: utilising tannery waste as a protein source for poultry feed, in: 28th International Conference on Solid Waste Technology and Management. The Journal of Solid Waste Technology and Management, Philadelphia.

Paul, H.L., Antunes, A.P.M., Covington, A.D., Evans, P., Philips, P.S., 2013. Bangladeshi leather industry: An overview of recent sustainable developments. J. Soc. Leather Technol. Chem. 97, 25–32.

Peng, L., Yi, J., Yang, X., Xie, J. and Chen, C., 2023. Development and characterization of mycelium bio-composites by utilization of different agricultural residual byproducts. Journal of Bioresources and Bioproducts, 8(1), pp.78-89.

PETA, 2020a. The Leather Industry [WWW Document]. URL https://www.peta.org/issues/animals-used-for-clothing/leather-industry/ (accessed 3.20.20).

PETA, 2020b. Silk, Cashmere, Shearling, and Other Animal-Derived Clothing [WWW Document]. URL https://www.peta.org/issues/animals-used-for-clothing/animals-used-skins/ (accessed 3.25.20).

PETA, 2019. Leather: Animals Abused and Killed for Their Skins [WWW Document]. URL https://www.peta.org/issues/animals-used-for-clothing/animals-used-clothing-factsheets/leather-animals-abused-killed-skins/ (accessed 3.20.20).

Petter, O., 2019. Is “Vegan Leather” Really More Sustainable Than The Real Thing? Indep.

Pelletier, M.G., Holt, G.A., Wanjura, J.D., Greetham, L., McIntyre, G., Bayer, E. and Kaplan-Bie, J., 2019. Acoustic evaluation of mycological biopolymer, an all-natural closed cell foam alternative. Industrial Crops and Products, 139, p.111533.

Piccin, J.S., Gomes, C.S., Mella, B. and Gutterres, M., 2016. Color removal from real leather dyeing effluent using tannery waste as an adsorbent. Journal of Environmental Chemical Engineering, 4(1), pp.1061-1067.

Piez, K.A., Eigner, E.A., Lewis, M.S., 1963. Collagen Structure alpha helices. Biochemistry 2, 58–66.

Pimentel, D., Pimentel, M., 2006. Global environmental resources versus world population growth. Ecol. Econ. 59, 195–198. https://doi.org/10.1016/j.ecolecon.2005.11.034

Pinatex [WWW Document], 2020. URL https://www.ananas-anam.com/ (accessed 3.20.20).

Pithers, E., 2019. The Leather Debate: Is Vegan Leather A Sustainable Alternative To The Real Thing? Vogue.

Gooch, J.W. 2007 (eds) Encyclopedic Dictionary of Polymers. Poromeric. Springer, New York, NY. https://doi-org.manchester.idm.oclc.org/10.1007/978-0-387-30160-0_9138

Priebe, G.P.S., Gutterres, M., 2017. Special review: Anaerobic digestion of leather industry wastes - An alternate source of energy. J. Am. Leather Chem. Assoc. 112, 59–71.

Prince, M., 2010. Chouwara Tannery, Fez [WWW Document]. Flickr under CC BY 2.0. URL https://www.flickr.com/photos/mikeprince/4781400829/in/photostream/ (accessed 6.29.20).

Purcell, B., Forgacs, G., 2017. Fibre Reinforced Tissue Composites. WO 2017/053433 A1.

Qiang, T., Han, M., 2018. The microstructure of raw hides of crocodylus niloticus. J. Soc. Leather Technol. Chem. 102, 169–173.

Qiang, T.T., Chen, X.K., Wang, X.C., Ren, Y.Q., Zhou, G.X., Yang, S.Q., 2012. Biodegradation of chrome-free goat garment leathers. J. Soc. Leather Technol. Chem. 96, 56–59.

Qu, J.B., Zhang, C.B., Feng, J.Y., Gao, F.T., 2008. Natural and synthetic leather: A microstructural comparison. J. Soc. Leather Technol. Chem. 92, 8–13.

Ramachandran, G.N., Bansal, M., Bhatnagar, R.S., 1973. A hypothesis on the role of hydroxyproline in stabilizing collagen structure. Biochim. Biophys. Acta 332, 166–171.

Ramachandran, G.N., Kartha, G., 1954. Collagen triple helical structure. Nat. 174, 269–270.

Ramachandran, G.N., Ramakrishman, C., 1976. Molecular Structure of Collagen, in: Ramachandran, G.N., Reddi, A.H. (Eds.), Biochemistry of Collagen. Plenum, New York, pp. 45–84.

Ramasami, T., 1996. Greening of chrome tanning in Indian leather industry. ILIFO J Clean. Tann. 1, 12–14.

Randall, D., Mandy, D.Y.M., 2004. Fish Physiology, Toxicology, and Water Quality, in: Eight International Symposium Chongping, China, October 2004. Ecosystems Research Division, Georgia, p. 155.

Rangel-Serrano, A., Maldonado-V, M., Kösters, K., 2003. Characterization of waste materials in tanneries for better ecological uses. J. Am. Leather Chem. Assoc. 98, 43–48.

Rao, J.R., Prakash, A., Thangaraj, E., Sreeram, K.J., Saravanabhavan, S., Nair, B.U., 2008. Natural dyeing of leathers using natural materials. J. Am. Leather Chem. Assoc. 103, 68–75.

Rastogi, S.K., Kesavachandran, C., Mahdi, F., Pandey, A., 2007. Occupational cancers in leather tanning industries: A short review. Indian J. Occup. Environ. Med. 11, 3–5. https://doi.org/10.4103/0019-5278.32456

Salvagnin, U., 2008. Cell Culture [WWW Document]. Flickr under CC BY 2.0. URL https://www.flickr.com/photos/34745138@N00/3075268200 (accessed 6.30.20).

Saravanabhavan, S., Thanikaivelan, P., Rao, J.R., Nair, B.U., Ramasami, T., 2004. Natural Leathers from Natural Materials: Progressing toward a New Arena in Leather Processing. Environ. Sci. Technol. 38, 871–879. https://doi.org/10.1021/es034554o

Sathish, M., Sathya, R., Aravindhan, R., Rao, J.R., 2017. Successful use of remnant: Attractive reversible grain pattern leather from bovine ear. J. Am. Leather Chem. Assoc. 112, 1–6.

Saxena, G., Purchase, D., Mulla, S.I. and Bharagava, R.N., 2020. Degradation and detoxification of leather tannery effluent by a newly developed bacterial consortium GS-TE1310 for environmental safety. Journal of Water Process Engineering, 38, p.101592.

Seal, B.L., Otero, T.C., Panitch, A., 2001. Polymeric biomaterials for tissue and organ regeneration. Mater. Sci. Eng. R Reports 34, 147–230. https://doi.org/10.1016/S0927-796X(01)00035-3

Senel-Ayaz, H.G., Har-El, Y. El, Ayaz, H., Lelkes, P.I., 2018. Textile technologies for 3D scaffold engineering, in: Deng, Y., Kuiper, J. (Eds.), Functional 3D Tissue Engineering Scaffolds: Materials, Technologies, and Applications. Woodhead Publishing, UK, pp. 175–201. https://doi.org/10.1016/B978-0-08-100979-6.00008-2

Shankar, A., 2014. Fascinating & Toxic - Traditional Moroccan Tanneries [WWW Document]. Living on Earth. URL http://www.loe.org/shows/segments.html?programID=14-P13-00044&segmentID=4 (accessed 7.2.20).

Sharkey, L., 2019. Stella McCartney & Adidas' New Sustainable Collection May Offer The Solution To Fashion's Waste Problem. Bustle.

Sharphouse, J.H., 1995a. What is Leather?, in: Leather Technician's Handbook. Leather Producers' Association, Northampton, UK, pp. 3–4.

Sharphouse, J.H., 1995b. Supply of Raw Hides and Skins to the Tannery, in: Leather Technician's Handbook. Leather Producers' Association, Northampton, UK, pp. 5–7.

Sharphouse, J.H., 1995c. Types of Hides and Skins and Principal Uses, in: Leather Technician's Handbook. Leather Producers' Association, Northampton, UK, pp. 20–24.

Sharphouse, J.H., 1995d. Types of Hides and Skins and Principal Uses, in: Leather Technician's Handbook. Leather Producers' Association, Northampton, UK, pp. 27–33.

Sharphouse, J.H., 1995e. Outline Recipes, in: Leather Technician's Handbook. Leather Producers' Association, Northampton, UK, pp. 482–513.

Sharphouse, J.H., 1995f. Flaying and Curing, in: Leather Technician's Handbook. Leather Producers' Association, Northampton, UK, pp. 8–19.

Sharphouse, J.H., 1995g. Unhairing and Liming, in: Leather Technician's Handbook. Leather Producers' Association, Northampton, UK, pp. 96–113.

Sharphouse, J.H., 1995h. Tanning Processes, in: Leather Technician's Handbook. Leather Producers' Association, Northampton, UK, pp. 144–233.

Sharphouse, J.H., 1995i. Fatliquoring of Light Leathers, in: Leather Technician's Handbook. Leather Producers' Association, Northampton, UK, pp. 322–339.

Sharphouse, J.H., 1971. Pollution, in: Leather Technician's Handbook. Leather Producers' Association, Northampton, UK, pp. 452–469.

Sheie, R., 2013. Leather goods sourced from Buenos Aires [WWW Document]. Flickr under CC BY 2.0. URL https://www.flickr.com/photos/85546319@N04/8525441785 (accessed 6.30.20).

Siegrist, M., Sütterlin, B., Hartmann, C., 2018. Perceived naturalness and evoked disgust influence acceptance of cultured meat. Meat Sci. 139, 213–219. https://doi.org/10.1016/j.meatsci.2018.02.007

Siggel, L., Bulo, R.E., Molnar, F., Weiss, H., Taeger, T., 2007. Leather related collagen modeling: The challenges of modeling hierarchical structures. J. Am. Leather Chem. Assoc. 102, 333–336.

Singh, J., Carlisle, D.L., Pritchard, D.E., Patierno, S.R., 1998. Chromium-induced genotoxicity and apoptosis: Relationship to chromium carcinogenesis (review). Oncol. Rep. 5, 1307–1318.

Sivan, A., 2011. New perspectives in plastic biodegradation. Curr. Opin. Biotechnol. 22, 422–426. https://doi.org/10.1016/j.copbio.2011.01.013

Sivasubramanian, S., Manohar, B.M., Puvanakrishnan, R., 2008. Mechanism of enzymatic dehairing of skins using a bacterial alkaline protease. Chemosphere 70, 1025–1034. https://doi.org/10.1016/j.chemosphere.2007.07.084

Sizeland, K.H., Wells, H.C., Edmonds, R.L., Kirby, N., Haverkamp, R.G., 2016. Effect of tanning agents on collagen structure and response to strain in leather. J. Am. Leather Chem. Assoc. 111, 391–397.

Sorolla, S., Jorba, M., Marginet, X., Castell, J.C., Ollé, L.L., Bacardit, A., 2010. Design of scaffolds for biopelt from tanning sector by-products. J. Soc. Leather Technol. Chem. 94, 190–199.

Specht, E.A., Welch, D.R., Rees Clayton, E.M., Lagally, C.D., 2018. Opportunities for applying biomedical production and manufacturing methods to the development of the clean meat industry. Biochem. Eng. J. 132, 161–168. https://doi.org/10.1016/j.bej.2018.01.015

Spencer, C.M., Cai, Y., Martin, R., Gaffney, S.H., Goulding, P.N., Magnolato, D., Lilley, T.H., Haslam, E., 1988. Polyphenol complexation-some thoughts and observations. Phytochemistry 27, 2397–2409. https://doi.org/10.1016/0031-9422(88)87004-3

Sreeram, K.J., Ramasami, T., 2003. Sustaining tanning process through conservation, recovery and better utilization of chromium. Resour. Conserv. Recycl. 38, 185–212. https://doi.org/10.1016/S0921-3449(02)00151-9

Stella McCartney, 2020a. Sustainability: Vegetarian Leather [WWW Document]. URL https://www.stellamccartney.com/experience/en/sustainability/themes/materials-and-innovation/vegetarian-leather/ (accessed 3.20.20).

Stella McCartney, 2020b. Sustainability: Silk [WWW Document]. URL https://www.stellamccartney.com/experience/en/sustainability/themes/materials-and-innovation/silk/ (accessed 3.20.20).

Stephens, N., Ellis, M., 2020. Cellular agriculture in the UK: a review. Wellcome Open Res. 5, 1–16. https://doi.org/10.12688/wellcomeopenres.15685.1

Stern, N., 2007. The economics of climate change: The stern review, The Economics of Climate Change: The Stern Review. Cambridge University Press, Cambridge. https://doi.org/10.1017/CBO9780511817434

Stockman, G., Didato, D.T., Hurlow, E., 2007. Antibiotics in hide preservation and bacterial control. J. Am. Leather Chem. Assoc. 102, 62–67.

Subramanian, A., Krishnan, U.M., Sethuraman, S., 2011. Skin tissue regeneration, in: Bosworth, L., S.Downes (Eds.), Electrospinning for Tissue Regeneration. Woodhead Publishing Limited, India, pp. 298–316. https://doi.org/10.1016/B978-1-84569-741-9.50014-8

Sundar, J. and Muralidharan, C., 2019. Total salinity elimination during preservation of animal skins: a sustainable approach through benign alternatives. Environmental Science and Pollution Research, 26, pp.32891-32901.

Supp, D.M., Boyce, S.T., 2005. Engineered skin substitutes: Practices and potentials. Clin. Dermatol. 23, 403–412. https://doi.org/10.1016/j.clindermatol.2004.07.023

Tahiri, S., De La Guardia, M., 2009. Treatment and valorization of leather industry solid wastes: A review. J. Am. Leather Chem. Assoc. 104, 52–67.

Tasca, A.L., Puccini, M., 2019. Leather tanning: Life cycle assessment of retanning, fatliquoring and dyeing. J. Clean. Prod. 226, 720–729. https://doi.org/10.1016/j.jclepro.2019.03.335

Taylor, M.M., Bailey, D.G., Feairheller, S.H., 1987. REVIEW OF THE USES OF ENZYMES IN THE TANNERY. J. Am. Leather Chem. Assoc. 82, 153–165.

Telemeco, T.A., Ayres, C., Bowlin, G.L., Wnek, G.E., Boland, E.D., Cohen, N., Baumgarten, C.M., Mathews, J., Simpson, D.G., 2005. Regulation of cellular infiltration into tissue engineering scaffolds composed of submicron diameter fibrils produced by electrospinning. Acta Biomater. 1, 377–385. https://doi.org/10.1016/j.actbio.2005.04.006

Thomas, D., 2019. Fashionopolis: The Price of Fast-Fashion and the Future of Clothes. Apollo, London.

Threads, B., 2019. Bolt Technology: Microsilk [WWW Document]. URL https://boltthreads.com/technology/microsilk/ (accessed 3.20.20).

Tokatli, N., 2008. Global sourcing: Insights from the global clothing industry - The case of Zara, a fast fashion retailer. J. Econ. Geogr. 8, 21–38. https://doi.org/10.1093/jeg/lbm035

Tucker, B., 2017. Leather Insights: A Comprehensive Guide for Buying the Best Leather [WWW Document]. Des. Tips, Octane Seat. URL https://www.octaneseating.com/leather-buying-guide (accessed 5.6.19).

Tu, Y.H., Ahn, M., Rakonjac, J., Holmes, G. and Norris, G., 2022. Milk provides the basis for an eco-friendly shorter process for skin preservation and leather manufacture. Cleaner Engineering and Technology, 8, p.100464.

Tulloh, N.M., 1961. Variations in the skin and skin-fold thickness of beef cattle. Aust. J. Agric. Res. 12, 992–1004. https://doi.org/10.1071/AR9610992

Tuomisto, H.L., 2019. The eco-friendly burger. EMBO Rep. 20, 1–6. https://doi.org/10.15252/embr.201847395

Tuomisto, H.L., Teixeira De Mattos, M.J., 2011. Environmental impacts of cultured meat production. Environ. Sci. Technol. 45, 6117–6123. https://doi.org/10.1021/es200130u

Vale, W.G., 2010. Deep freezing buffalo semen - state of art. Rev. Vet. 21, 844–855.

Valeika, V., Beleška, K., Biškauskaitė, R., Valeikienė, V., 2019. Effect of enzymatic soaking on properties of hide and the leather produced. J. Soc. Leather Technol. Chem. 103, 74–79.

Van-Eelen, W.F., Van-Kooten, W.J., Westerhof, W., Mummery, C.L., 2006. Industrial Production of Meat. US 2006/0029922 A1.

Van Der Valk, J., Mellor, D., Brands, R., Fischer, R., Gruber, F., Gstraunthaler, G., Hellebrekers, L., Hyllner, J., Jonker, F.H., Prieto, P., Thalen, M., Baumans, V., 2004. The humane collection of fetal bovine serum and possibilities for serum-free cell and tissue culture. Toxicol. Vitr. 18, 1–12. https://doi.org/10.1016/j.tiv.2003.08.009

VanBenschoten, J.J., Tasset, D.G., Eversole, R., Kallenberger, W.E., 1985. Production Of White Leather and Boil-stable Brown Leather Using Titanium. J. Am. Leather Chem. Assoc. 80, 237–242.

Vedaraman, N., Muralidharan, C., 2011. Two stage leather dyeing - A novel approach to minimize the dye discharge in the effluent. J. Am. Leather Chem. Assoc. 106, 184–189.

Vedaraman, N., Sandhya, K. V., Brindha, V., Tamil Selvi, A., Velappan, K.C., Muralidharan, C., 2016. Recovery, purification and reuse of contaminated sodium chloride obtained from tanneries for raw goat skin preservation. J. Am. Leather Chem. Assoc. 111, 62–68.

Vein, J., 2004. Method for Producing Tissue Engineered Meat for Consumption. US 6835390 B1.

Venugopal, J., Low, S., Choon, A.T., Ramakrishna, S., 2008. Interaction of cells and nanofiber scaffolds in tissue engineering. J. Biomed. Mater. Res. - Part B Appl. Biomater. 84, 34–48. https://doi.org/10.1002/jbm.b.30841

Venugopal, N.B.R.K., Reddy, S.L.N., 1992. Nephrotoxic and hepatotoxic effects of trivalent and hexavalent chromium in a teleost fish Anabas scandens: Enzymological and biochemical changes. Ecotoxicol. Environ. Saf. 24, 287–293. https://doi.org/10.1016/0147-6513(92)90004-M

Walker, M.P., Webster, R.M., Bugby, A., Alexander, K.T.W., 1990. Early detection of grain faults during wet processing. J. Am. Leather Chem. Assoc. 85, 191–211.

Waltz, E., 2022. Bio-leather gears up to wow fashion industry. Nature Biotechnology, 40(3), pp.287-289.

Wang, L., Fan, X., Sun, Y., Sun, X., 2014. Effects of fibre pore status on hot-wet comfort of sheep garment leather. J. Soc. Leather Technol. Chem. 98, 113–120.

Wang, Y., Kim, H.J., Vunjak-Novakovic, G., Kaplan, D.L., 2006. Stem cell-based tissue engineering with silk biomaterials. Biomaterials 27, 6064–6082. https://doi.org/10.1016/j.biomaterials.2006.07.008

Watt, A., 1906. Chrome Leather Manufacture, in: Leather Manufacture. Crosby Lockwood and Son, London, pp. 323–347.

Williams, E., Cenian, K., Golsteijn, L., Morris, B. and Scullin, M.L., 2022. Life cycle assessment of MycoWorks' Reishi™: the first low-carbon and biodegradable alternative leather. Environmental Sciences Europe, 34(1), p.120.

Wilson, J.A., 1941a. Hides and Skins and their Histology, in: Modern Practice in Leather Manufacture. Reinhold Publishing Corporation, New York, pp. 15–58.

Wilson, J.A., 1941b. Vegetable Tanning Materials and Their Properties, in: Modern Practice in Leather Manufacture. Reinhold Publishing Corporation, New York, pp. 272–305.

Wilson, J.A., 1941c. Chrome Tanning, in: Modern Practice in Leather Manufacture. Reinhold Publishing Corporation, New York, pp. 363–411.

Wu, J., Zhao, L., Liu, X., Chen, W., Gu, H., 2017. Recent progress in cleaner preservation of hides and skins. J. Clean. Prod. 148, 158–173. https://doi.org/10.1016/j.jclepro.2017.01.113

Wunderlich, S. and Gatto, K.A., 2015. Consumer perception of genetically modified organisms and sources of information. Advances in nutrition, 6(6), pp.842-851.

Yaman, R., 2020. Cellular/Acellular Agriculture Patent Landscape [WWW Document]. URL https://docs.google.com/spreadsheets/d/1l3PLthSMdEnU6Gn8_3phVe5h5XcktgZYa_rA7G-DJyk/edit#gid=2080497194 (accessed 4.27.20).

Yang, S., Leong, K.F., Du, Z., Chua, C.K., 2001. The design of scaffolds for use in tissue engineering. Part I. Traditional factors. Tissue Eng. 7, 679–689.

https://doi.org/10.1089/107632701753337645

Yeh, C., Perng, D.B., 2001. Establishing a Demerit Count Reference Standard for the Classification and Grading of Leather Hides. Int. J. Adv. Manuf. Technol. 18, 731–738.

Yilmaz, O., Cem Kantarli, I., Yuksel, M., Saglam, M., Yanik, J., 2007. Conversion of leather wastes to useful products. Resour. Conserv. Recycl. 49, 436–448. https://doi.org/10.1016/j.resconrec.2006.05.006

Yousefi, N., Jones, M., Bismarck, A. and Mautner, A., 2021. Fungal chitin-glucan nanopapers with heavy metal adsorption properties for ultrafiltration of organic solvents and water. Carbohydrate Polymers, 253, p.117273.

Zapletal, P., 1997. Technological value of hides of crossbreeds from Black-and-White cows with beef bulls of Piemontese, Chianina and Marchigiana breeds. J. Soc. Leather Technol. Chem. 81, 224–226.

Zhou, H., Tan, Z., Li, X., 2012. Assessment of wastewater pollution in pig leather industry in China. Water Environ. J. 26, 521–529. https://doi.org/10.1111/j.1747-6593.2012.00312.x