

## Review Article

# Recent developments in sustainably sourced protein-based biomaterials

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Research into the development of sustainable biomaterials is increasing in both interest and global importance due to the increasing demand for materials with decreased environmental impact. This research field utilises natural, renewable resources to develop innovative biomaterials. The development of sustainable biomaterials encompasses the entire material life cycle, from desirable traits, and environmental impact from production through to recycling or disposal. The main objective of this review is to provide a comprehensive definition of sustainable biomaterials and to give an overview of the use of natural proteins in biomaterial development. Proteins such as collagen, gelatin, keratin, and silk, are biocompatible, biodegradable, and may form materials with varying properties. Proteins, therefore, provide an intriguing source of biomaterials for numerous applications, including additive manufacturing, nanotechnology, and tissue engineering. We give an insight into current research and future directions in each of these areas, to expand knowledge on the capabilities of sustainably sourced proteins as advanced biomaterials.

## Introduction

Sustainable biomaterials refer to the development of innovative biomaterials using building blocks obtained from natural, renewable resources. Due to their unique properties and abundance, proteins hold great promise as a green source of sustainable biomaterials for various applications. Additionally, alternative protein sources, such as recombinant proteins and peptides produced in different prokaryotic, eukaryotic, plant, and mammalian expression systems have also been explored to develop biomaterials with properties comparable to natural materials. This review provides an overview of sustainable biomaterials and the potential of protein biomaterials within this context. It covers aspects of protein sourcing and processing, along with recent trends and challenges, including alternative sustainable protein sources for biomaterial development, followed by examples of different protein-based biomaterials and their properties and applications.

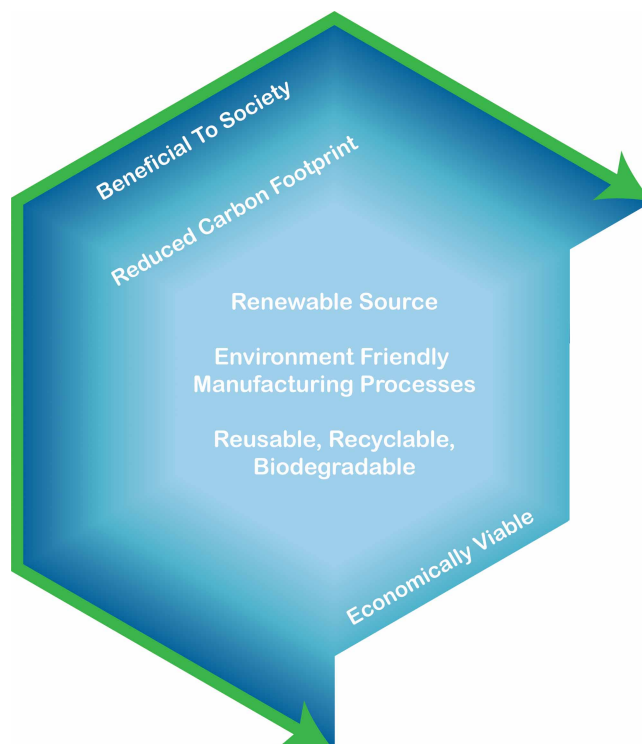
## Sustainable biomaterials

To understand what a sustainable biomaterial is, the entire life cycle of the material needs to be considered. This includes desirable and durable material characteristics, and the environmental impact of the entire production process, from the production through processing, degradation, recycling, or ultimate disposal at the end of its useful lifespan [1–3]. Based on these factors, a sustainable biomaterial can be classified as a material that is

- Sourced from sustainably grown renewable resources.
- Produced using environmentally friendly manufacturing processes.
- Safe and non-hazardous for the environment.
- Reused and recycled at the end of their intended use, such as feedstock for another process or via recycling or composting.

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**Figure 1. Sustainability criteria for materials, from environment related factors, to economy-related factors and social contexts; these concepts are interconnected. Adapted with permission from [1]. Copyright 2016 by Taylor and Francis.**

Other important aspects to consider for sustainability criteria are economic feasibility and societal benefits. For instance, in considering environmental impact, a sustainable biomaterial should fulfil a real need of society so that the ecological footprint of its production can be justified by its benefits to the society [1]. For this review, these social-economic issues will not be discussed in detail, but it is important to highlight that their integration is vital as shown in Figure 1. This will improve the sustainability outcomes of the new generation of biomaterials and to contribute to achieving the United Nation's sustainable development goals for 2030 [4].

Based on the sustainability criteria above, protein-based biomaterials can be classified as sustainable materials due to their abundance and renewability [5,6], and ease of modification and chemical heterogeneity, offered by the variable amino acid composition of proteins obtained from different sources [7,8]. Furthermore, as most proteins are water-soluble, the entire production process can be conducted with limited use of organic solvents [9]. Protein biomaterials also offer exceptional physical properties, with the enormous benefit that they are intrinsically biodegradable making them a part of a circular economy of source materials [10]. There is, therefore, currently much interest in both developing new protein-based materials and sourcing proteins for these materials in different ways [6,11–13].

## Protein sourcing and processing for sustainable protein biomaterials

A great diversity in protein-based biomaterials with various applications in different fields exists, however, to meet sustainability criteria it is important that the protein source is renewable and the entire production process including biomaterial development is low energy and eco-friendly [14]. In this context, the use of proteins from agro-wastes or food side streams provides a sustainable, economical, and reliable feedstock [15]. Similarly, the direct utilisation of several fibrous proteins, such as keratin, silk, resilin, and elastin into biodegradable hierarchical biomaterials also offers sustainable methods towards developing green biomaterials [10,16].

In the last few years, several proteins have emerged as novel sustainable protein biomaterials due to their easy availability, simple production methods and biocompatibility, for example, soy [17,18], milk proteins —

casein [19,20], whey [21,22], keratin [23], collagen [24,25], haemoglobin [26], silk [27,28]. However, there are some drawbacks to harvesting these proteins from natural sources, these include batch-to-batch variation, impurities, the quantity available, the risk for disease transmission, and potentially fast biodegradation [29,30]. To mitigate these issues, biotechnological solutions have been developed to produce larger amounts of protein with more consistent quality and greater biological safety [8,11,31,32].

## Alternative protein sourcing technologies

Recombinant technology provides an alternative method to produce proteins that are not naturally available in abundant quantities, as well as to modify the properties of these recombinant proteins, for example, by adding other bioactive molecules, growth factors, cell-binding domains, or other direct chemical modifications [33,34]. There are many successful reports of using of recombinant proteins to develop protein-based biomaterials; however, issues relating to low yield and scalability hinder their widespread commercial adoption [35–37]. However, with increasing interest in cellular agriculture, these limitations may soon be overcome. Table 1 provides a non-exhaustive list of different proteins used to develop biomaterials, along with their commonly used source and alternative sources available. For a more in-depth discussion on the recombinant production of proteins towards sustainable biomaterials, the authors refer readers to detailed reviews on this subject [54–57]. In the following sections: additive manufacturing, nanotechnology and tissue engineering, a range of applications of protein-based biomaterials will be discussed.

## Additive manufacturing

Additive manufacturing, also called three-dimensional (3D) printing, has been recognised as a more sustainable manufacturing method compared with traditional manufacturing, allowing for flexible designs and prints of complex structures [58]. The advantageous energy and environmental impacts of additive manufacturing is thoroughly reviewed in [59]. Current 3D printing research explores the integration of natural biopolymer resources such as proteins, polysaccharides and other green materials as the main components of novel bio- and biomaterial inks [60].

Groll et al. [61] propose a general definition of bio-inks that clarifies its distinction from biomaterial inks. They define a bio-ink as ‘a formulation of cells suitable for processing by an automated biofabrication technology that may also contain biologically active components and biomaterials’ which differs from biomaterial inks which do not contain cells during processing — cells can be post-seeded onto the end-biomaterial product depending on the applications. The development of such inks is well reviewed in many recent publications [61–67].

Collagen, gelatin, silk fibroin, and keratin are examples of proteins that are employed in the formulation of inks for a variety of applications such as films, hydrogels and scaffolds for tissue, bone and cartilage engineering [57,62,68]. Features such as biocompatibility and biodegradability along with the ease of availability, low processing cost, and varying mechanical properties make proteins ideal components of biomaterial inks [67]. Protein-based inks along with the additive manufacturing process offer an opportunity to address the niche issue of tissue engineering — e.g. fabrication of suitable scaffolds with biocompatibility and functional mechanical properties. The layer-by-layer assembly provides a complex 3D construct with robust and highly ordered

**Table 1. Proteins used to develop biomaterials, along with their commonly used source and alternative sources available**

Protein	Traditional source	Alternative source
Keratin	Wool fibres, horns, nails and feathers from butchery [23,38–40].	Biomimetic recombinant hagfish thread keratin [41], recombinant human hair keratins K31 and K81 [42,43].
Collagen	Collagen type I: Mammalian skin, and tendon tissues (porcine, bovine and ovine in origin); collagen type II: bovine, porcine and chicken cartilaginous tissues [24,44]; marine collagen [45–47].	Recombinant human collagen in different prokaryotic, eukaryotic, plant and mammalian expression systems [35,48].
Silk	Domestic silkworms: <i>Bombyx mori</i> ( <i>B. mori</i> ); wild silkworms: <i>Antheraea pernyi</i> and <i>Samia cynthia ricini</i> ; Spiders: <i>Nephila clavipes</i> and <i>Araneus diadematus</i> [12].	Recombinant silk proteins in different host systems [11,29,49].
Elastin and resilin	Animal-derived tropoelastin, recombinant production [16].	Recombinant elastin-like recombinamers (ELRs) [11,36,39]; Recombinant resilin-like peptides (RLPs)/ protein [50–52] or protein [53].

structures for cell proliferation and differentiation [69]. Major classes of printing processes where proteins are used in are lithography and material extrusion [70,71]. Choi et al. [57] review the advances in protein-based materials and the following is a snapshot of how proteins are integrated within bio- and biomaterial inks.

## Protein-based inks

Collagen materials are abundant throughout biomaterials research, and Marques et al. [72] present a comprehensive review on the state-of-the-art of the development of collagen-based inks for tissue engineering applications. Various methods to improve the printability of collagen by either combining collagen with synthetic polymers or by directly printing collagen into a sacrificial support gel have been established [73,74]. For example, a homogeneous hybrid collagen/nano-hydroxyapatite formulation has been developed to mimic the composition of bone tissue with suitable properties for 3D printing via material extrusion processes [73]. Gelatin, formed from the partial hydrolysis of collagen, is also frequently used in the biomaterial industry. Modifying gelatin with methacrylic anhydride (MA) to obtain gelatin-methacryloyl (GelMA) is the most common modification of the protein to render it photosensitive, and protocols are well established throughout the literature [75–77]. Compared with the more solid collagen/nano-hydroxyapatite ink that is extruded, GelMA is a moderately viscous liquid ink that solidifies upon exposure to ultraviolet light. GelMA is used in various applications such as micropatterning, fluidic systems, 3D scaffolds and bioprinting [75,78–81]. GelMA is also added to synthetic polymer mixtures for improved mechanical properties. One example is the addition of GelMA to methacrylated poly(vinyl alcohol) (PVA-MA) with tris-bipyridyl ruthenium hexahydrate and sodium persulfate (Ru/SPS) as the photoinitiator [82]. This study describes the development and characterisation of a bio-resin containing PVA-MA/Gel-MA/Ru/SPS which demonstrated compatibility with commercial 3D printing fabrication using a vat photopolymerisation process [82].

Silk fibroin-based scaffolds have also been fabricated using 3D printing processes. Mixtures of silk with several other biopolymers such as gelatin and alginate were developed to achieve the rheological requirements suitable for 3D extrusion printing [83]. The methacrylation of silk fibroin favours the 3D printing of silk via lithography techniques. One recent study from Kim et al. [65] follows a similar approach to GelMA production, where silk fibroin is methacrylated using glycidyl methacrylate to print scaffolds via digital light processing 3D printing. They also demonstrated the cytocompatibility of the Sil-MA hydrogels by successfully encapsulating NIH/3T3 fibroblast within the hydrogels [65]. And, more recently, Grigsby et al. [38] published a paper on the fabrication of keratin-lignin inks for 3D printing using green chemistry principles. Keratin is one such protein, which can be extracted from wool, feathers, and other biological waste products from the meat industry, a sustainable, economical, and reliable feedstock [84–86]. In this case, keratin is extracted as an intermediate filament protein powder by treating wool with copper sulfate and sodium sulphite following a standard oxidative sufitolysis procedure. Keratin-lignin copolymer materials show promising capability for processing as filaments in material extrusion printing (more specifically, fused deposition melting, FDM, printing) and for deposition as hydrogels using 3D paste printing [38].

The literature demonstrates a wide range of successful research outcomes for the use of natural proteins within the additive manufacturing sector. The flexibility in transforming proteins into different sorts of inks, from viscous photosensitive liquids to hard melting filaments, allows for the fabrication of a large variety of biomaterials. The layer-by-layer construction of these biomaterials offer a particular advantage for the fine detail of the constructs and specific seeding of cells which may be difficult to obtain from traditional manufacturing such as moulding. Overall, the combined use of protein-based inks and 3D printing technologies offer an opportunity for an improved and more sustainable production of biomaterials.

## Building blocks for nanotechnology

Nanotechnology and the creation of new nanostructures offer many exciting opportunities for new products and materials. However, there are concerns regarding the environmental impact of both the processes to produce nanostructures and the nanostructures themselves which have led to the adoption of green chemistry techniques for nanostructure synthesis. Proteins are one of nature's nanostructures, self-assembling from amino acids into a range of intriguing nanoscale structures, such as nanofibrils and nanocages. Protein nanostructures are of great interest as biomaterials, due to their increased biocompatibility when compared with more traditional nanomaterials, e.g. metallic nanoparticles [87]. They have shown the potential to be used in extensive applications, such as biosensors, catalysts, and drug delivery systems [88–91]. Here, we will provide some key examples of protein nanostructures, specifically protein nanofibrils, nanoparticles, and nanogels. For further

reading on the use of protein nanotechnology, including the use of recombinant technology to produce designed protein nanostructures, we refer the readers to [92].

## Protein nanofibrils

Beta-sheet rich protein nanofibrils, or amyloid-like fibrils, have many desirable properties for use in biomaterial fabrication. When compared with other natural fibrous structures, like collagen fibrils, amyloid-like fibrils have been found to have distinct advantages in regards to tensile strength, stability, and deformation resistance, with many different proteins able to adopt this structure [93]. For instance, whey proteins (a by-product of cheese-making) can form amyloid-like protein nanofibrils, at low pH ( $\leq 2$ ), low ionic strength, and high temperature ( $\geq 80^\circ\text{C}$ ), and the resulting structures are observed to be highly stable under various environmental [94]. These fibrils alone can make different forms of materials, such as hydrogels (via the addition of divalent cation salts), microcapsules (via a layer-by-layer self-assembly technique), and coatings (via adsorption) [95–97].

With the further addition of other biomolecules, such as nanoparticles, enzymes, and bioactive substances, whey protein nanofibrils (WPNFs) can display other functions and forms. For example, WPNFs can induce the formation of  $\text{ZrO}_2$  nanoparticles from Zr ions, and a fluoride ion-removal filter membrane can be produced using these nanoparticle-carrying fibrils and a carbon matrix via vacuum-filtration, which exhibited excellent selectivity for F-against various competitive ions [98]. Moreover, WPNFs may contribute interesting features to a new composite material. Zhang et al. [99] demonstrated an emulsion microstructure formed with WPNFs, oil and D-limonene that showed excellent antioxidant and antibacterial activity and functions as a carrier system for lipophilic bioactive molecules. When composited with graphene, the presence of  $\beta$ -lactoglobulin (from whey protein isolate) fibrils endowed unique periodic troughs and crests to the topography of the composite film without the need of lithographic techniques or etching, enabling this film to provide an efficient support for cellular polarisation and differentiation [100]. Later, the same fibrils were mixed with silica precursor tetraethyl orthosilicate, and fibril-silica core-shell nanostructures with stiffness up to beyond  $\sim 20$  GPa (similar Young's moduli as many metal alloys and inorganic materials) were made [101]. In fact, many proteins can be reconstructed into this particular structure under certain conditions, including many sustainably sourced proteins, such as casein, haemoglobin (from meat industry waste), crystallins (from fish industry waste), and soy proteins [94,102–105]. However, compared with whey protein fibrils, less research has focused on these alternative nanofibril sources as biomaterials. Future biomaterials design should take advantage of the flexible choice of protein fibrils according to their specific intended application.

## Nanoparticles (NPs)

NPs generally refer to materials with sizes ranging from 1 to 100 nm [106]. Collagen-based ZnO NPs have been successfully fabricated into hexagonal wurtzite structure with particle sizes ranging between 20 and 50 nm by a bioinspired synthesis method [107]. These NPs not only exhibited antibacterial, antibiofilm, and anti-cancer (against human liver (HepG2) cells) effects, but also showed no ecotoxicity [107]. Another method to produce nanoparticles using sustainably sourced proteins was demonstrated by Perotto and co-workers. They extracted keratin from wool and formed keratin particles by water-solving the auxiliary structure [40]. The tunable mucoadhesive properties of these particles can dramatically improve drug delivery efficiency [40].

Bioactive glass (bioglass) has been developed to supplement damaged bone tissue in the physiological environment. Reiter and colleagues coated bioactive glass ( $\text{SiO}_2$ -CaO- $\text{P}_2\text{O}_5$  system) nanoparticles with gelatin by using a foam replication technique [108]. With the addition of gelatin, the scaffold's strength and toughness were significantly increased. Additionally, the cross-linked gelatin network endowed the bioglass with the ability to sustained release the bioactive molecule icariin for bone defect repair [108].

## Other nanostructures

Different from typical hydrogels, nanohydrogels/nanogels (NGs) are nano-scaled cross-linked networks showing both features of a hydrogel and nanoparticle [109]. Kang and colleagues used fish gelatin to form NGs, which can contain and release doxorubicin by pH regulation, through water-in-oil emulsion and photopolymerization [110]. This nano-sized drug delivery system shows many advantages, including enhanced permeability, retention (EPR) effect, and improved drug stability. Nanonets commonly compromise interlinked ultrathin fibres which are stacked layer-by-layer in the final porous membranes [111]. Wang et al. [111,112] demonstrated spider-web-like nanonets ( $\sim 35$  nm) structures formed by gelatin through electrospinning and a



recent paper based on their work designed more complicated systems to improve the magnetic properties of  $\text{CoFe}_2\text{O}_4$  nanoparticles.

In addition to the known advantages of nanoscale structures, in the examples detailed, protein nanostructures are favourable due to their ease of synthesis, and often improved functional properties, particularly biocompatibility.

## Tissue engineering

The application of biomaterials to tissue engineering represents a logical progression of protein-based technology. The tunable physical, biological, and chemical functionalities of proteins provide a toolbox from which materials for almost any tissue engineering application can be designed. Here, we have very crudely delineated our examples into drug delivery vehicles or structural scaffolds. However, these are generalised terms under which a variety of subclasses such as medical devices, biosensors, and tissue mimics can be defined.

## Drug delivery vehicles

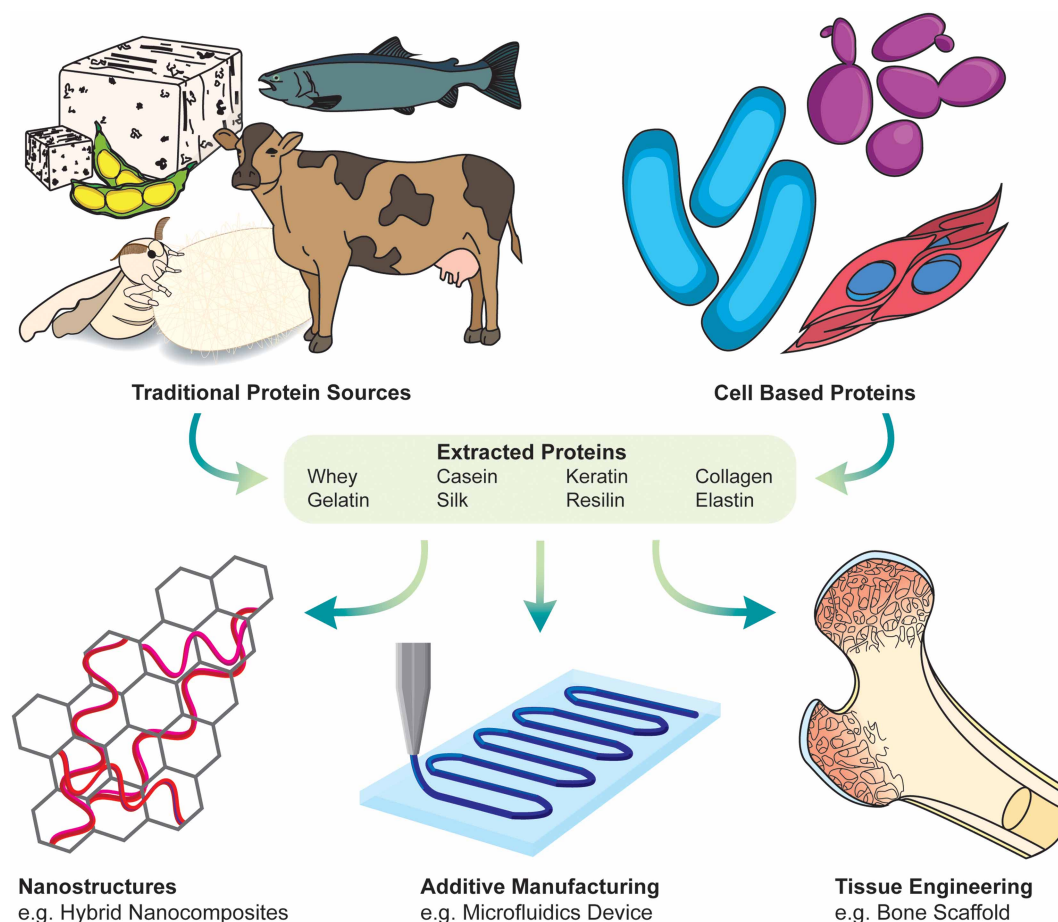
Much like the nanostructures discussed earlier, protein biomaterials can be utilised for drug delivery. Bioengineered silk offers a divergence from traditional *Bombyx mori* reliant production and allows for functionalisation for targeted applications. A prime example is the production of silk spidroin proteins with the integration of the H2.1 or DOX peptide [113]. H2.1 selectively binds to Human Epidermal growth factor Receptor 2 (HER2), a receptor expressed by some aggressive forms of breast cancer, while DOX confers an affinity for the cancer drug doxorubicin. Composite silk spidroin spheres, therefore, offer a cancer drug delivery system which is targeted, controlled and biodegradable [113], potentially enhancing patient outcomes and reducing overall medical costs. Bioengineered silk is more sustainable than traditional silk harvesting, with recombinant systems being designed in prokaryotic and eukaryotic models including *Escherichia coli*, rice, tobacco, and kidney cells [114]. If green production and processing of silk fibroin and spidroin become commonplace, the use of silk biomaterials for drug delivery could be utilised for consumer products. For example, improving insulin delivery patient compliance is of particular interest in translational medicine. Subcutaneous injections of silk fibroin hydrogels with encapsulated insulin enables sustained release of insulin for blood glucose control in diabetic rats [115]. Similarly, silk fibroin microneedle patches offer a sustained release of insulin via transdermal delivery, removing the need for traditional injections which require high patient compliance and careful management of injection sites to protect tissue integrity [116].

Collagen biomaterials also exhibit potential in drug delivery, having natural biocompatibility as an extracellular matrix protein [44]. Collagen extracted from turkey tendon, a waste product from the poultry industry, can be formed into sponge scaffolds following gelation, cross-linking and freeze-drying, with prilocaine hydrochloride loaded as a model drug [44]. Gelatin, formed from the partial hydrolysis of collagen, can similarly form hydrogels for drug delivery applications. Hydrogels formulated from gelatin and hyaluronic acid produce an injectable substrate which directly delivers anti-inflammatory drugs to knee joints for the treatment of osteoarthritis [117]. Gelatin/salcan (a bacterial polysaccharide) composites loaded with vancomycin, an antibiotic traditionally given intravenously, showed sustained release over several days and positive preliminary results for eliminating *E. coli* and *Staphylococcus aureus* populations in co-culture [118]. Delivery vehicles enhance the longevity, targeting, and therapeutic efficacy of pharmaceuticals, making biodegradable protein-based scaffolds a viable strategy for improving patient outcomes.

## Structural scaffolds

Protein scaffolds are of particular interest in the tissue engineering field for their biocompatibility, mechanical properties, and biodegradability. Keratin is one such protein, and its ability to be formed into a rigid, yet porous, scaffold makes it well suited for bone matrix engineering. Wool keratin scaffolds demonstrate chemical and physical stability and can be manufactured by both liquid casting [119] and electrospinning with polymers [120]. Both methods produce porous scaffolds supporting cell migration, adhesion and expression of osteogenic markers. The biocompatibility of keratin scaffolds is such that bone grafts have been shown not to elicit innate or antibody-mediated immune responses in ovine models, a critical feature for any scaffold intended for implantation [121].

Elastin is an extracellular matrix protein commonly sourced from bovine neck ligaments. As the name suggests, elastin is a protein which confers elasticity and resilience to tissue. The inclusion of elastin cross-linking into tendon-derived collagen scaffolds designed for vascular grafts both improves the mechanical properties and increases endothelial and smooth muscle cell viability [122]. Hydrogels and films produced from



**Figure 2. Illustrative representation of renewable protein sources and their potential application in the biomaterials field.**

genetically engineered elastin expressed in *E. coli* have been formulated to specifically promote endothelial cell adhesion [39], a property which if combined with the aforementioned vascular grafts could improve vascular tissue engineering technology. Elastin has also been shown to improve the mechanical properties of other protein-based materials, such as gelatin and cellulose acetate scaffolds for skin tissue engineering where the ability of the graft to flex with surrounding healthy tissue is paramount [123]. Resilin, much like elastin, is a protein with high elasticity and resilience to repetitive mechanical loading [50]. Although natively found in insects, resilin and modified resilin-like proteins are commonly produced by expression in bacterial culture [50–52]. Resilin-like proteins can be designed to include function-specific peptides to mimic tissue and promote differentiation of cells [50]. Structurally, engineered resilin can form conductive, elastic and adhesive hydrogels suitable as biosensors [52], or be combined with silk spidroin carboxyl-terminal domains to increase the gelation temperature range of reversible hydrogels [51]. Whether a product uses elastin or resilin, derived from industry waste material or modified and recombinantly produced, will depend on specific material requirements. However, both offer improved properties for tissue mimics in which resilience is necessary for successful integration.

Recombinant peptides based on human type I collagen can be produced by yeast fermentation [124]. Following methacrylation and formation into hydrogels (as detailed in Protein-based inks section), it displays comparable properties to hydrogels formed from animal-derived methacrylated gelatins to mimic extracellular matrix and support encapsulated stem cells [124]. Bacterial fermentation is also being utilised, with human-like collagen expressed in *E. coli* BL21 being used in conjunction with the polysaccharide chitosan to produce hydrogels which accelerate the closure of full-thickness skin defects [125]. However, when vast natural sources of collagen are available, recombinant protein production is perhaps less renewable. Chicken skin collagen electrospun with elastin displays similar promise for the development of engineered skin substitutes [126].

Products derived from marine environments, so-called ‘blue biomaterials’, are also on the rise. Collagen extracted from eel [45], codfish [46], and tilapia [47] skin all exhibit biocompatibility and structural properties comparable with traditional, mammalian collagen sources.

In this paper, we have covered a non-exhaustive list exemplifying just some of the potential applications that materials formed from sustainably sourced proteins have in the biomaterials field, as illustrated in Figure 2. Despite great opportunity, there remain multifaceted challenges in designing fully sustainable and efficient protein-based biomaterials. For example, batch-to-batch variation, and sometimes complicated paths of translation to clinical settings. We expect future work to address these challenges, as well as uncover new novel protein sources and material fabrication techniques.

## Perspectives

- Fabricating biomaterials from natural polymers such as proteins, offer various advantages such as increased biocompatibility, biodegradability, cytocompatibility, and flexible construct designs. This field is leading in the development of innovative materials and more sustainable production of biomaterials by the efficient harnessing of natural resources.
- Currently, the development of protein-based biomaterials is expanding our knowledge on the importance of using available natural resources to improve the negative environmental impacts of some traditional materials and to increase the valorisation of these natural resources.
- As large scale fermentor technology improves, cellular agriculture is likely to become a more common source of producing protein biomaterials.

## Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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## Author Contribution

All authors reviewed the literature, selected and organised content, and wrote major text sections. J.G. prepared the figures. L.J.D. and H.A. critically revised the manuscript.

## Abbreviations

GelMA, gelatin-methacryloyl; NGs, nanogels; NPs, nanoparticles; WPNFs, whey protein nanofibrils.

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