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Chicken egg white: Hatching of a new old biomaterial

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Chicken egg white is an abundant, inexpensive and natural source of important proteins such as ovalbumin and lysozyme. Thanks to its bioactivity, easy handling, anti-bacterial activity and biodegradability, egg white is being used since centuries as excipient of poultices for the treatment of various disorders. Owing to unique thermal and electrical features, egg white is currently used in bioplastic development and in fabrication of field-effect transistors, but it could also contribute to various biomedical applications in the future. Indeed, egg white and some of its byproducts were shown to improve tissue engraftment and to stimulate angiogenesis, making it particularly attractive in wound healing and tissue engineering applications. Moreover, egg white can be manipulated to obtain versatile platforms for tridimensional *in vitro* tissue models or drug delivery systems. This review describes the structure and physicochemical properties of egg white as well as its biological features. It also summarizes fabrication methods from egg white for the generation of functional platforms, and provides a comprehensive overview of the role and performance of egg white in various biomedical applications. Finally, new perspectives for future studies in health with this ancient material are critically discussed.

Introduction

Although ceramic and metallic biomaterials are widely used in biomedical applications, polymeric materials attract great attention due to the versatility of their chemistry, manufacturability, tunable physico-mechanical properties and degradation rates. Among them, natural polymers, which are classified as proteins (*e.g.* collagen), polysaccharides (*e.g.* alginate) or polynucleotides (*e.g.* DNA), owing to their intrinsic bioactivity and biodegradability have been preferred over synthetic polymers in various biomedical studies [1]. Natural biomaterials mimic extracellular matrix (ECM) elements and present peptide sequences, protease cleavage or integrin cell-binding sites which in turn, results in enhanced cellular behavior both *in vitro* and *in vivo* [2–4]. When enhanced mechanical properties are required, natural polymers are often used in combination with synthetic polymers [5,6].

Among different natural polymers available for biomedical applications, those with high bioactivity and availability, easy handling and low production costs are of great interest. Although polysaccharides are used in various applications as injectable cell carriers or three dimensional (3D) printing bioinks, they lack certain cell-binding sites, which limits their biological activities [7,8]. Protein-based biomaterials are bioactive and support cellular responses such as attachment, proliferation and migration, but they often either have limited sources or require complex extraction and purification procedures, which makes them costly or inaccessible [9]. Interestingly, egg white is a low cost and easily available protein-based material directly

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usable in its raw form for different applications. Moreover, thanks to its intrinsic liquid phase, no additional extraction or resuspension into a solution are needed and its viscosity can also be modulated by temperature. In addition, high transparency of egg white renders it suitable for 3D cell culture systems through which cell growth can be easily monitored [10,11]. Besides, owing to its bioactive components, egg white offers a wide variety of biological activities such as wound healing, cell growth promotion and anti-bacterial properties [12].

Chicken egg white, an excellent natural source of proteins, is an overlooked native biomaterial with eminent physicochemical, structural and biological properties. The history of egg white dates back to the ancient times (before 1000 AD), when egg white has been used by Egyptian, Roman and Persian physicians to treat multiple disorders [13–15]. Based on what is available today from ancient handwritten and manuscripts, egg white has been implemented as poultice, cataplasm or ointment for wound healing purposes, particularly as burn dressing, as well as cancer treatment. Egg white alone or mixed with honey, cabbage, or herbs has been prescribed to soothe inflamed eyes and remedy the sprains and swellings, and to cover the fractured limbs [16–18]. While these evidences indicate the medicinal benefits of egg white, the underlying molecular mechanisms are yet to be better understood. Although egg white has been overlooked during the past decades, an increasing amount of work has been recently reported on biological applications of egg white, thanks to its antibacterial, healing-enhancing, antihypertensive, anti-inflammatory, and cell growth stimulatory features. In light of its growing involvement in new areas of biomedical sciences, a detailed review on the structure, biological properties and biomedical applications of egg white is still required. This review consolidates and discusses the general properties, different morphologies and current applications of egg white-based materials, as well as the future prospects for their further developments in biomedical science and engineering.

Egg white structure and physicochemical properties

The major structural components of chicken egg are shell, shell membrane, yolk and white (Fig. 1a). Egg shell, which is mainly composed of calcium and phosphate, has a porous structure (~17,000 tiny pores) that allows air permeation to the interior. Eggshell membrane, with protein-based fibrous structure, resides between the egg shell and white, and supports the formation of enzymes and proteins. Eggshell membrane that mimics the ECM in human tissues, has three morphologically distinct layers; outer, inner and limiting membranes that together, protect the egg contents from bacteria (Fig. 1a) [19–21]. Egg yolk suspended in the egg white via two connection tissues, named chalazae, feeds the developing embryo as such, yolk is a great source of vitamins and nutrients. Despite having high amount of cholesterol (~11 mg/g of edible portion) and lipids, serum yolk works as a reservoir for large quantities of hen's immunoglobulin (IgY), which could be used as an alternative source of antibodies for prevention and treatment of infectious diseases [22–24].

Egg white, also known as "albumen", is mainly a mixture of water (~85%), proteins (~10%) and carbohydrates (~5%), and acts as a second protection layer to prevent penetration of bacteria to the yolk. The composition of egg white as compared to whole egg has been reviewed in Table 1. Egg white is composed of four layers that differ in viscosities, and named based on their viscosity and position in respect to yolk: outer thin (next to the shell membrane), outer thick, inner thin and inner thick (chalaziferous) layers. Presence of high content of ovomucin in thick portions results in their high viscosity (40 times greater than thin portions). Rheological behavior of the whole egg white is most similar to that of the thin portion and it shows pseudoplastic properties, where its apparent viscosity decreases with increasing temperature until the fluidity is lost at around 60 °C [25]. Moreover, during shearing, the filamentous super aggregates of thick parts break down, leading to lower viscosities observed in thin portions [26].

Egg white's multifunctional features (such as gelling, foaming, water-binding and emulsifying) make it a great material for the food industry as well as biomedical applications. Some of the most relevant egg white's physical and structural properties have been summarized in Table 2. The emulsifying activity and emulsion stability of egg white proteins, which affects their functionality, are dependent on pH, protein concentration and presence of salts. The surface hydrophobicity of ovalbumin, main protein of egg white, is greatest at pH 3. Thus, reducing the pH of the egg white solution to 3 maximizes its emulsifying activity without changing the secondary structure and globular conformation of proteins [27]. By exploiting the hydrophobic–hydrophobic interactions between egg white protein chains at pH 3, hydrogels can be fabricated and used as bioactive materials for tissue engineering applications and beyond [28]. Since residues of hydrophobic amino acids are mainly located within the globular protein molecules, extra processing steps, such as liquification, thermal treatment or pH change, are often prerequisite for establishing intermolecular interactions. Otherwise, the internal electrostatic and hydrophobic interactions, and covalent disulfide intramolecular bonds restrain the molecular flexibility of egg white proteins [29].

Egg white protein composition

As principal elements of egg white, proteins importantly contribute to its physical and biological properties [30]. Egg white proteins are globular and categorized into two groups: main proteins (more abundant, >83% of total proteins; Fig. 1b) and minor proteins (less abundant, <17%) [31,32], as described below. In the following paragraphs, some of the proteins more often involved in bioengineering are introduced and ordered based on their abundance.

Main proteins

Ovalbumin

Ovalbumin (OVA) is a phosphoglycoprotein which comprises 54% of total egg white proteins [32]. The full-length sequence of OVA contains 385 amino acids with a molecular weight of 44.5 kDa and an isoelectric point (p*I*) of 4.5 [33]. The OVA structure is predominantly formed from 41% of α-helix, 34% of β-sheet, 12% of β-turns as well as 13% of random coils [34]. The protein chain of OVA ends with acetylated glycine and proline amino acids in the N- and C-terminals, respectively [35]. One OVA molecule contains 4 free sulfhydryl groups, 1 single disul-

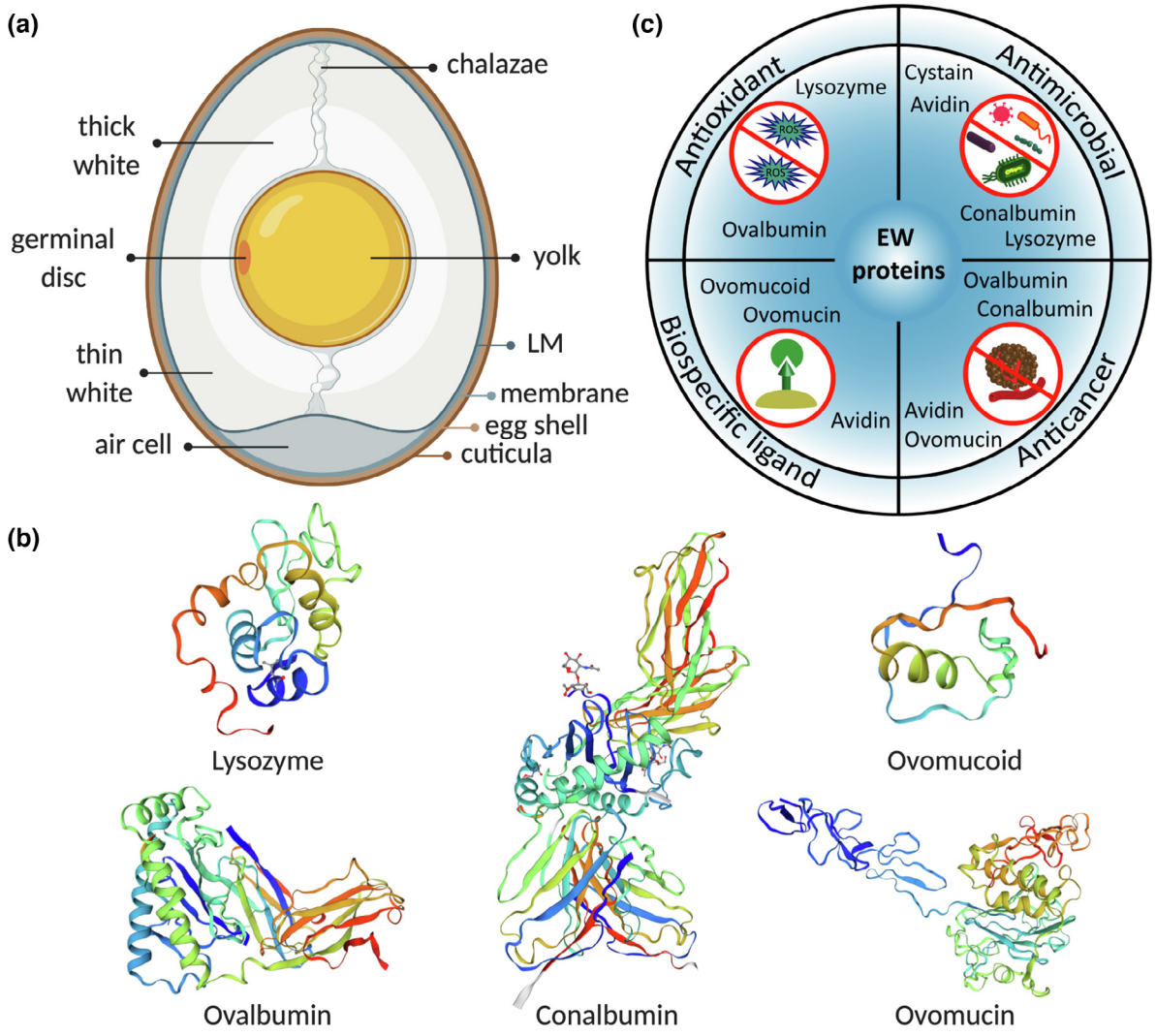

FIGURE 1

(a) Major components of chicken egg are shell, shell membrane, yolk and white. Egg white is located between eggshell membrane layers (*i.e.* outer, inner and limiting (LM) membranes) and yolk (created with Biorender.com). (b) 3D structure of five main egg white proteins; ovalbumin, conalbumin, lysozyme, ovomucoid and ovomucin. (c) Egg white proteins exhibits different biological properties, making them ideal bioactive compounds for multiple medical, pharmaceutical and bioengineering areas.

fide bond, 0–2 phosphoryl groups and one carbohydrate chain in an OVA molecule [36,37]. Owing to its unique surface and thermal properties, OVA plays a pivotal role in heat-induced gelation, foaming and emulsifying properties of egg white [38–40]. However, it is noteworthy that the structure and properties of OVA can change over storage time. For instance, the percentages of α-helix and β-sheet are diminished, while the percentage of β-turns and random coils are increased [40]. Moreover, a proportion of OVA is irreversibly converted into a more heat-stable form, S-ovalbumin, which is contingent upon temperature, time and pH [29,41].

Conalbumin

Conalbumin, also known as ovotransferrin, is a glycoprotein which accounts for 13% of total protein content in egg white [32]. This protein is composed of 686 amino acids with a molecular weight of 77.9 kDa and a pI of 6 [42]. The polypeptide chain of conalbumin is folded into two lobes (N- and C-lobes) that are interconnected by an α-helix [43]. Each lobe includes two domains linked by antiparallel β-strands and owes a strong binding site for iron and other transition metals such as copper, zinc and aluminum [32–44]. Conalbumin has 15 disulfide bonds (6 in N-lobe and 9 in C-lobe) [45] and one glycan chain linked to C-terminal domain [46]. These disulfide bonds provide the structural stability [45] and the iron binding capability endows conalbumin with antimicrobial properties [29,47]. Conalbumin is the most heat-sensitive protein, undergoing denaturation and aggregation at 53–65 °C and leading to alteration of the egg white viscosity and initial gelation [48–50]. However, OVA is able to inhibit the thermal aggregation of conalbumin at a temperature lower than its own denaturation temperature of OVA [50].

Ovomucoid

Ovomucoid (OM) is a glycoprotein forming 11% of total proteins in egg white [32]. With 186 amino acid residues in its chain, OM has a molecular weight of 28 kDa and a pI of 4.1 [51]. Its structure

TABLE 1

Composition of whole egg and egg white [226,227].

Component (per 100 g)	Whole egg (100%)	Egg white (58%)
Water (g)	74.4	88.6
Proteins (g)	12.3	10.6
lipids (g)	11.9	0.1
Carbohydrates (g)	0.7	0.8
Minerals (mg)		
Na	120	155
Cl	172	175
K	125	140
Ca	50	8
P	193	18
Fe	1.7	0.1
Mg	12	10
S	164	163
Zn	1.4	0.12
Cu	0.06	0.02
Mn	0.04	0.007
I	0.05	0.003
Vitamins (mg)		
Vitamin A	150	0
Vitamin B1	913	10
Vitamin B2	447	430
Vitamin B6	133	10
Vitamin B12	1	0.1
Vitamin D	1.5	0
Vitamin E	1200	0
Folic acid	56	12
Niacin	79	90
Biotin	25	7
Pantothenic acid	1700	250
Essential amino acids (mg)		
Isoleucine	290	240
Leucine	660	560
Lysine	1040	880
Methionine + Cystine	820	660
Phenylalanine + Tyrosine	640	670
Threonine	1150	1020
Tryptophan	590	470
Valine	190	170

consists of 46% of β-sheet, 10% of β-turns and 26% α-helix along with 18% of random coils [32]. Moreover, the molecule of OM is divided into three domains, each of which contains 60 amino acids and is crosslinked by three intradomain disulfide bonds, however, no disulfide bridge exists between domains [52]. OM functions as a trypsin inhibitor [53]. Furthermore, it is also recognized as a prominent allergen in egg white because of its strong resistance to heat and enzymatic digestion and its allergic reactivity [54,55].

Ovomucin

Ovomucin is another glycoprotein which is found in egg white, contributing to 3.5% of proteins [32]. It is arranged in two subunits: α-subunit and β-subunit linked by disulfide bonds [56]. α-Subunit contains a lower level of carbohydrates (15%) and has a molecular weight of 210 kDa, while β-subunit with a molecular weight of 5500–8300 kDa is rich in carbohydrate (60%) [57,58]. As a highly viscose protein that confers the gel-like structure to the egg white, ovomucin acts as a mechanical barrier for egg yolk against pathogens [59]. It is also thermally stable and has the tendency to interact with other proteins [60].

Lysozyme

Lysozyme is a secretory enzyme which constitutes 3.4% of total proteins in egg white [32]. The single polypeptide chain of lysozyme contains 129 amino acids with a molecular weight of 14.4 kDa and a pI of 10.9 [61]. The three-dimensional structure of lysozyme is made up of two domains: N-domain consisting of antiparallel β-sheets and C-domain containing four α-helices [62–64]. The two domains are segregated by a helix–loop–helix motif which is situated at the upper side of the enzyme's active site [65,66]. Lysosome is cross-linked by 4 disulfide bonds which results in thermal stability as well as cohesion [35,60]. Being competent to catalyze the hydrolysis of peptidoglycan in cell walls of bacteria, lysozyme exhibits strong antimicrobial properties [67,68]. Furthermore, unlike other egg white proteins which possess negative charges at physiological pH, lysozyme is positively charged at this pH and therefore able to interact with negatively charged molecules [69,70].

Other proteins (minor proteins)

In addition to the main proteins, a number of other proteins are influential for the physiochemical and biological characteristics of the egg white, regardless of their limited abundance [71]. Nevertheless, not all the minor egg white proteins are fully identified and characterized, given that many of them are present with an extensive range of concentrations and molecular weights [11,72,73]. Ovoinhibitor (1.5%), ovoglycoprotein (1%), flavoprotein (0.8%), ovomacroglobulin (0.5%), cystatin (0.05%) and avidin (0.05%) are the most eminent among the minor proteins found in egg white [74]. Ovoinhibitor is a heat stable glycoprotein with 21 disulfide bonds, and it is competent to inhibit serine proteinase including trypsin, chymotrypsin, elastase and fungal proteinase [75–77]. Flavoprotein, also called ovoflavoprotein, is an acidic phosphoglycoprotein which has 8 disulfide bonds and can link to riboflavin (Vitamin B2) [33,78]. Cystatin, a small-sized protein with 2 disulfide bonds, displays thermostability, antibacterial properties as well as the ability to inhibit cysteine proteases such as papain and ficin [60,71,79]. Avidin is an alkaline, tetrameric glycoprotein which acts as an antibacterial agent [76,80] and possesses a very strong affinity for biotin (Vitamin B7) [81]. The avidin–biotin binding has been extensively employed for various applications such as immunoassays, gene probes, drug delivery, affinity chromatography and diagnostic assays [31,72]. Additionally, the Arg-Tyr-Asp-Ser (RYDS) sequence present in the avidin molecule is able to mimic the function of Arg-Gly-Asp-Ser (RGDS) sequence regulating the cell attachment [11].

Biological properties of egg white

Being rich in components with high biological activity, egg white exhibits some unique properties appealing for biomedical, pharmaceutical, nutraceutical, cosmetic and fodder purposes. Some biological activity of egg white includes anti-bacterial, cell attachment and growth, and growth factor binding properties [82].

Egg white proteins exert antibacterial effect via different mechanisms such as bacterial cell lysis, metal binding, or vitamin binding. Lysozyme, for instance, possesses inherent antibacterial features via hydrolyzing bacteria cell walls by breaking down the

TABLE 2

Physical and structural properties of egg white proteins [31,32,37,227,228].

Protein	Egg white (%)	MW (kDa)	pI	T_d (°C)	Structure	Disulfide bonds	Characteristics
Ovalbumin	54	45	4.5	84	Serpin-like structure with a three-turn α-helical reactive center loop	1	Heat-stable phosphoglyco-protein, emulsifying and foaming agent
Conalbumin	13	77.9	6.0	61	Two (N- and C-) lobes interconnected by an α-helix	15	Metal binding protein
Lysozyme	3.4	14.4	10.7	75	Two (N- and C-) domains segregated by a helix-loop-helix motif	4	Destruction of bacterial cell wall
Ovomucoid	11	28	6.1	70	Three domains	Intradomain: 9 (3 in each domain) Interdomain: 0	Trypsin inhibitor
Ovomucin	3.5	α: 210 β: 5500–8300	4.5–5.0	–	Two (α-and β-) subunits linked by disulfide bonds	+	Structural protein maintaining viscosity and structure of egg white
G2 globulin	4	36	5.0	92.5			Foaming agent
G3 globulin	4	45	4.8	–			Foaming agent
Ovoinhibitor	1.5	49	5.1	–	Seven domains	+	Serine protease inhibitor
Ovoglycoprotein	1	24.4	3.9	–	13.6% hexose, 13.8% hexosamine, and 3% sialic acid		Sialoprotein
Flavoprotein	0.8	32–36	4.0	–	Two domains: N-terminal with the riboflavin-binding site and C-terminal with a negatively charged amino acid portion	9	Riboflavin-binding protein
Ovomacroglobulin	0.5	650	4.5	–	Four subunits linked by disulfide bonds	+	Strongly antigenic protein, protease inhibitor
Cystatin	0.05	12.7	5.6 and 6.5	–	An α-helical region/a five stranded β-pleated sheet	2	Thiol protease inhibitor
Avidin	0.05	68	10	85	Four identical subunits	4 (1 in each subunit)	Biotin-binding protein

MW: Molecular weight.
pI: Isoelectric point.
T_d: Denaturation temperature.
+: Disulfide groups exist in protein.

β-1,4 linkage between the N-acetylglucosamine and the N-acetylmuramic acid of peptidoglycan barrier in bacteria [83]. Lysozyme inhibits the activity and growth of different strains of bacteria including Gram-positives (*e.g. Corynebacterium. glutamicum*) and Gram-negatives (*e.g. Escherichia coli* and *Shigella flexneri*). Although changes in viscosity and pH values modulate the antimicrobial activity of other egg white proteins, lysozyme retains its activity at both native and denatured states through enzymatic and nonenzymatic fashions, respectively [84]. Other proteins, such as Ovomucin, inhibit the spread of microorganisms by maintaining the structure and viscosity of egg white, thus constituting a physical barrier. Interestingly, besides its physical function, ovomucin possesses also owes *in vitro* antiviral activity against different viruses such as influenza [85,86]. Ovotransferrin, an indispensable antimicrobial component of egg's defense system, contains a distinct 92-amino acid domain within its N-lobe which permeates the outer membrane of Gram-negative bacteria, allows selective transfer of ions and eventually diminishes their cytoplasmic membrane [87]. In addition to their role in the intracellular catabolism of peptides and proteins, cystatins exert bactericidal activity and thus, are a potential candidate for antibacterial drug development [88].

Egg white proteins mediate cell adhesion and growth, and enhance their ability to establish interactions with biomaterials. Similar to glycoproteins, such as glycosidases, lectins and fibronectin, ovalbumin manifests high adhesion-mediating functions [89]. This adhesion-promoting feature is apparently cell-type specific, for instance, egg white was reported to significantly enhances the attachment and spreading of hamster fibroblasts (*e.g.* NIL and BHK cells) but not of liver cells. This property might be attributable to a certain recognition-adhesion reaction resulting from a surface activity peculiar to specific cell types [89,90], although the growth-promoting activity of egg white depends on dose and cell seeding density. Interestingly, egg white simulates the nerve growth factor (NGF) effects, by inducing the neurite outgrowth in mammalian cells (*e.g.* PC12 cells), suggesting the presence of NGF-like bioactive components in egg white [90]. In light of its various favorable biological properties, egg white has been used as a storage medium for keeping avulsed teeth viable for periodontal ligament (PDL) healing. Compared to other natural storage solutions, such as milk or human saliva, egg white resulted in higher PDL healing and lower amount of inflammatory, surface and replacement resorptions [91,92].

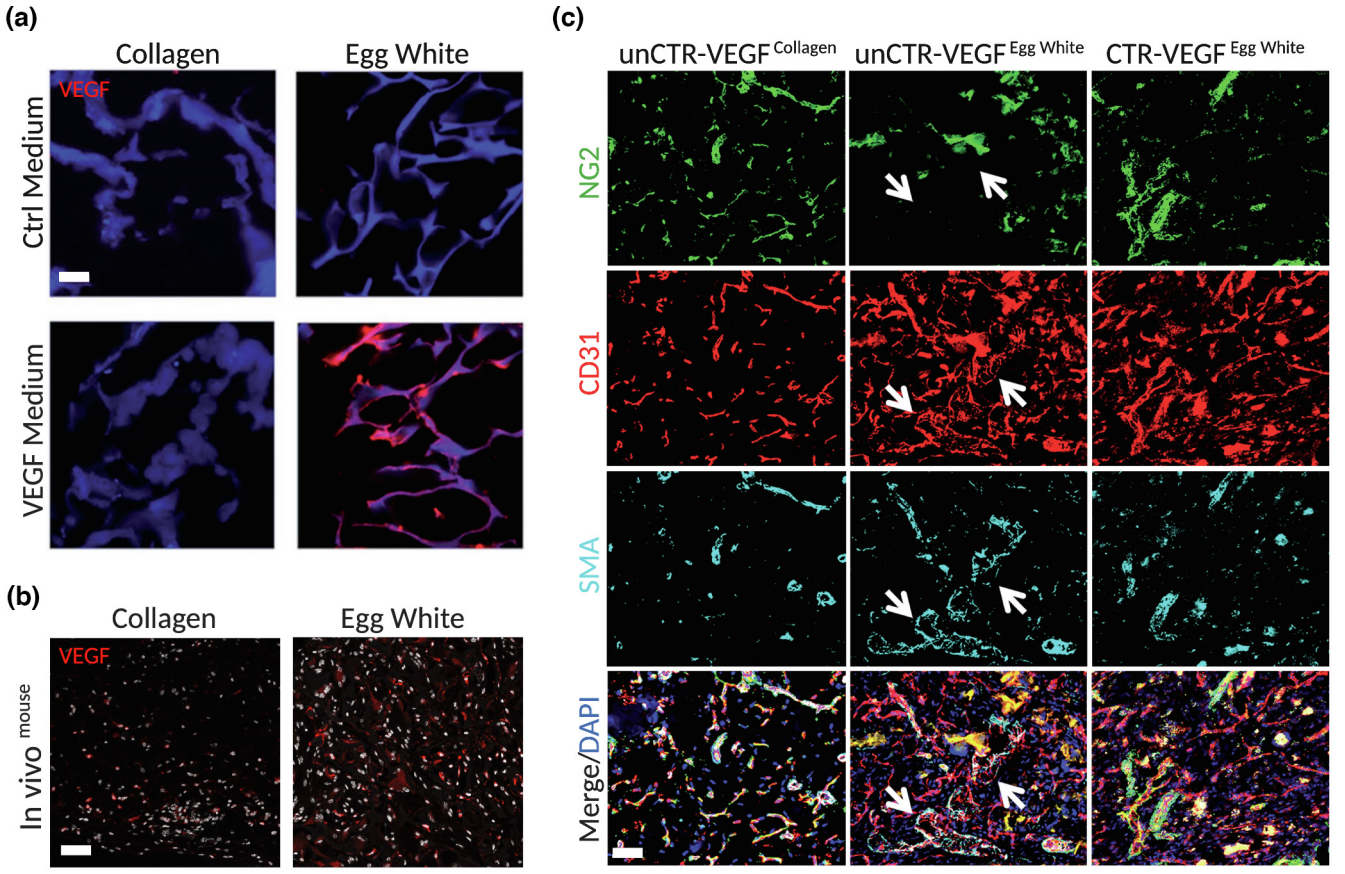

FIGURE 2

(a) *In vitro* retention of VEGF in egg white and collagen scaffolds (blue) in conventional cell culture medium and VEGF-supplemented medium; scale bar: 20 μm (figure adapted with permission from: Ref. [96]). (b) Immunofluorescence staining for VEGF in collagen and egg white sponges implanted subcutaneously in nude mice for 4 weeks shows a more abundant signal in egg white scaffold; scale bar: 50 μm (figure adapted with permission from: Ref. [28]). (c) Newly formed blood vessels induced by egg white scaffold seeded with adipose tissue-derived stromal cells (ASCs) expressing aberrant (unCTR) or controlled (CTR) VEGF levels after 14 days of implantation in an ectopic rat model; scale bar: 50 μm. Cell nuclei, pericytes, endothelial cells and smooth muscle cells are stained with DAPI, NG2, CD31, and SMA, respectively. White arrows identify aberrant (severely and heterogeneously enlarged) vessel structures (figure adapted with permission from: Ref. [96]).

Thanks to their protein composition offering anchoring sites for bioactive molecules, egg white sponges demonstrate increased cell attachment and proliferation *in vitro*, and enhance angiogenesis *in vivo* compared to other conventional sponges (Fig. 2a) [28]. Subcutaneous implantation of egg white macroporous sponges in nude mice revealed efficient cell colonization and engraftment of the construct with surrounding tissue with no evident inflammatory responses like fistula, infection or fibrous capsule. Immunohistochemical staining for CD31, a marker of endothelial cells, revealed that compared to collagen sponges with similar architecture and mechanical properties, egg white sponges displayed a substantial increase in the number, size and ingrowth depth of vessels (Fig. 2b). These features were associated with efficient cytokine adsorption and by enhanced polarization of macrophages into a regenerative, M2-like phenotype. M2 macrophages are known to improve tissue repair through elimination of interfering particles and cellular remnants, and by secreting cytokines and growth factors [28].

As a natural ECM, egg white is capable of adsorbing soluble growth factors, a property that not only increases their local concentration, but also modulates their diffusion, fine-tunes their concentration gradients, localizes their morphogenetic activity, enhances their biological activity and protects them from enzymatic degradation [28,93,94]. Angiogenesis is of great importance in clinical applicability of tissue engineering scaffolds, as it works to provide oxygen and nutrients within the tissues and prevent suboptimal tissue growth or cell death. The vascular endothelial growth factor (VEGF) is regarded as the major proangiogenic factor that regulates endothelial cells proliferation, migration and differentiation [95]. To adsorb and deliver VEGF, scaffolds like collagen sponges sometimes require chemical treatments for its covalent anchoring. Egg white, in contrast, intrinsically adsorbs VEGF upon *in vitro* incubation in VEGF-supplemented cell culture medium or following *in vivo* implantation in mouse and rat (Fig. 3a) [28,96]. When adipose tissue-derived stromal cells (ASCs) expressing aberrant VEGF levels were cultured on egg white-based sponges and implanted subcutaneously in rats, enlarged blood vessels with multiple lumens, covered with smooth muscle cells, were observed (Fig. 3b). In contrast, the same cell population on collagen sponges or ASCs expressing controlled levels of VEGF on egg white scaffold did not reveal enhanced angiogenesis [96]. This observation corrob-

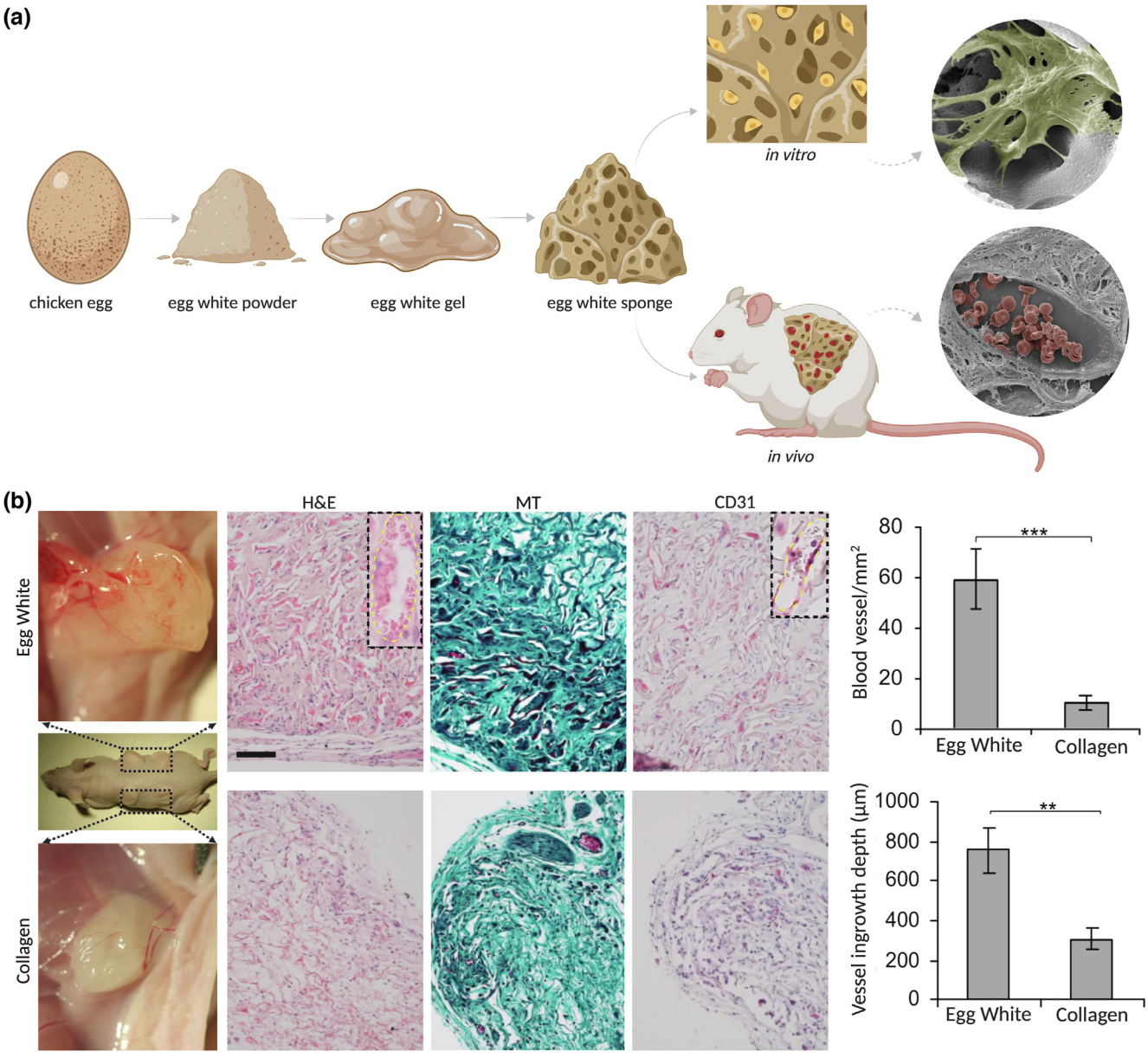

FIGURE 3

(a) Schematics of the processing egg white into a macroporous scaffold for *in vitro* and *in vivo* studies (created with Biorender.com). Scanning electron miroscopy images confirm the attachment of human dermal fibroblasts after 14 days of culture (cells are false colored for clarity; top right inset) and ingrowth of neo-vessels after 1 month implantation within the egg white scaffolds (erythrocytes are false-colored in red; bottom right inset) (electron microscopy images adapted with permission from: Refs. [28,225]). (b) (left panel) Interaction of collagen and egg white scaffolds with *in vivo* environment and angiogenesis evaluations. H&E images show the development of neovasculature in the scaffolds. The red color in images of CD31 represents the blood vessels. The insets show higher magnification of vessels. A more abundant and intense VEGF signal is observed in egg white as compared to collagen scaffold (Scale bar: 100 μm). (right panel) Counting of the blood vessels and their ingrowth depth in egg white and collagen scaffolds (**$P < 0.01$, ***$P < 0.001$) (figure adapted with permission from: Ref. [28]).

orates the superior capacity of egg white to adsorb large amounts of VEGF within its structure and improve angiogenesis in tissue engineering applications.

Morphological diversification of egg white-based materials

Due to easy supply and facile processing, egg white can generate a wide array of biomaterials, in which biotechnologies allow for control of mechanical properties and modulation of biological responses from seeded cells. In fact, the egg white proteins display versatile techno-functional features, such as foaming capability, emulsifying activity, and gel formation upon heating [97,98]. As a result of these processes, they can be shaped into porous scaffolds, hydrogels, films, fibers, particles, and nanogels (Fig. 4). Below, we review different morphologies of egg white biomaterials and summarize in Table 3 their advantages and limitations.

Macroporous sponges

With a configuration based on interconnected pores, sponges are ideal supporting materials for hosting cells and modeling vascularization processes [99,100]. Egg white-based macroporous sponges have exhibited excellent potential for soft tissue regeneration [28,101]. Importantly, the type and concentration of crosslinkers used in sponge fabrication are decisive factors in determining the overall scaffold characteristics, such as morphology, pore size, matrix degradation rate, denaturation temperature, and other mechanical features, without affecting the egg white bioactive properties [28,102]. For OVA sponge, crosslinking is often necessary to enhance its physico-mechanical attributes for both hard (*e.g.* bone) and soft (*e.g.* skin) tissue engineering applications [102,103], where cell proliferation and differentiation play pivotal roles to obtain optimal tissue regeneration.

Interestingly, the egg white itself can serve as foaming agent to produce desired pore size and porosity in polymeric and ceramic-based (*e.g.* calcium phosphate glass) constructs [104]. Benefiting from the foaming property of egg white, its individual proteins could also be mixed with other natural or synthetic polymers to exhibit enhanced bioactive behaviors. For example, ovomucin-based porous scaffolds were prepared by adding gelatin as a co-polymer to enhance the structure stability and protein foaming ability (Fig. 5a). When implanted subcutaneously into rats, the open porous design allowed cells to infiltrate into the matrix and more easily degrade the scaffold, and thus support tissue remodeling over time. The implanted sponge did not induce persistent fibrosis and only limited inflammatory and allergic responses appeared. Formation of blood vessels was also observed along the fibrous tissue formed at the periphery and within the central regions of ovomucin–gelatin sponge [101].

Under certain approaches, such as non-aqueous precipitation method and ultrasonication, sub-micrometric sponges can be developed. Recently, titanium/egg white composites were fabricated, which presented adsorption capability of organic pollutants such as methyl orange, high Brunauer–Emmett–Teller (BET) surface area, highly nanoporous structure, and exceptional photocatalytic activity [105].

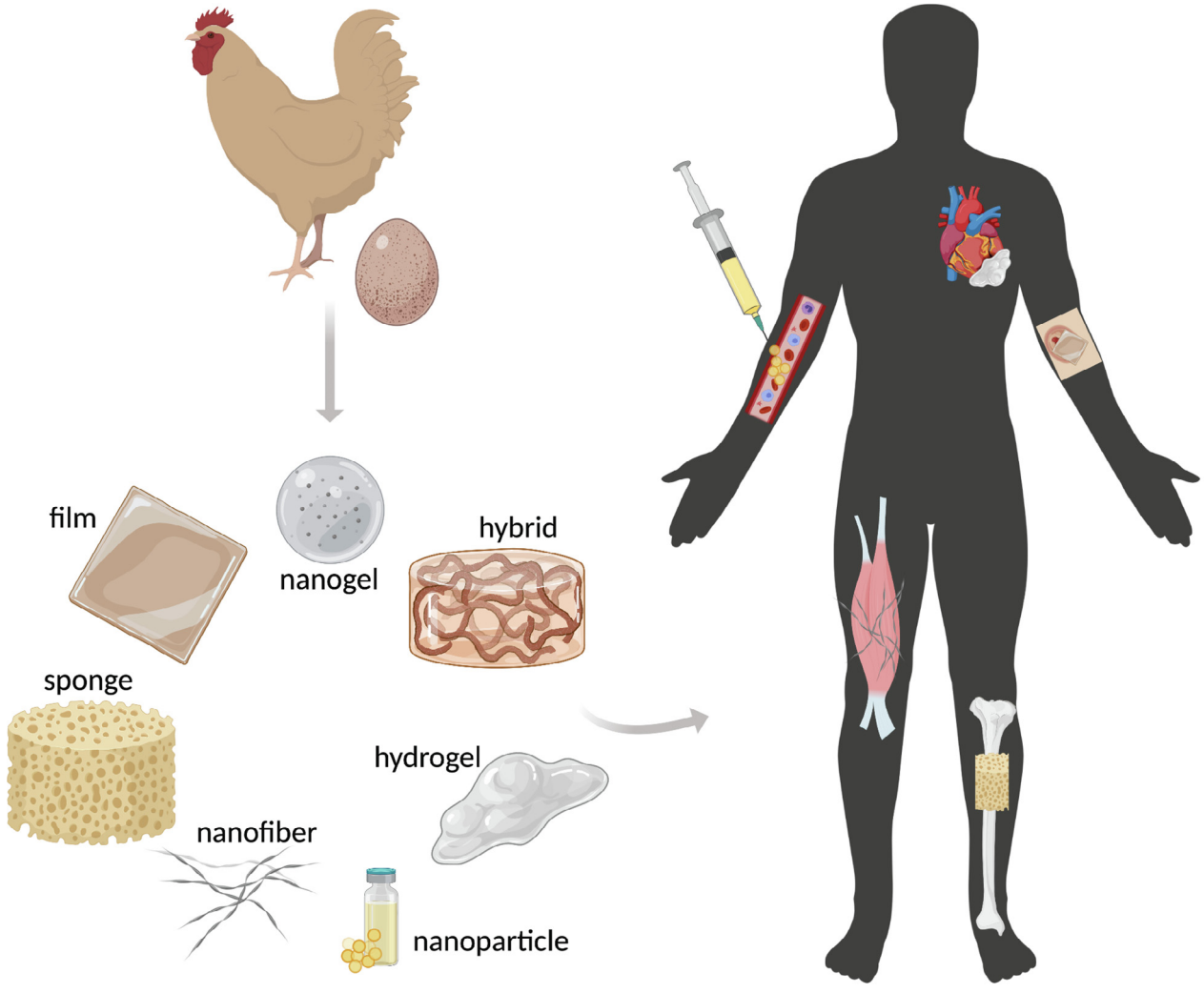

FIGURE 4

Schematic of processing of chicken egg white into different biomaterial forms and potential applications thereof. Egg white proteins could be solubilized with various solvents and once solubilized, could be processed into the range of different structures shown (created with Biorender.com).

TABLE 3

Main advantages and disadvantages of egg white biomaterials shaped in different morphologies.

Morphologies	Strengths	Limitations
Sponge 	High porosity enables cell–cell interactions and ECM deposition; Interconnective structure allows for nutrients/wastes transport and promotes tissue development; Prevent clustering of the cells, avoiding necrotic center formation	May shrivel; Different pore sizes are required for the specific cell types and are therefore time consuming
Film 	Enables multilayer construction; Transparent (allows wound healing monitoring); Facile fabrication processing	Not suitable for fabricating 3D complex structures; Limited mechanical (compression) properties; No cell infiltration
Hydrogel 	Soft and flexible; Multiple forms (transparent, opaque or particulate); Injectable and shear-thinning; Self-healing properties; Printable into complex 3D constructs	Not stable (uncontrolled dissolution may occur); Low mechanical resistance; Difficult to control the pore size
Nanofiber 	Their nanometric scale matches that of ECM fibers; Relatively large surface area beneficial for cell attachment and bioactive factor loading	Low mechanical properties; Fabrication process is challenging (bead formation); Limited cell infiltration
Microparticle 	Easily fabricated with controlled physical characteristics suitable for fast or sustained drug delivery; Targeted drug delivery	Batch-to-batch variation hinders the scaling-up process
Nanogel 	Prompt reaction to environmental stimulations; Reversible sites to bind and release drugs; Thermoreversible hydrophobicity and size change	Limited control over the nanogel size

Films

Films are defined as stand-alone thin layers of materials, that could be used as barriers, wraps or covers, in contrast to coatings which are layers formed *in situ* on a surface or substrate [106,107]. Amorphous films have been developed since decades from both whole egg white or its purified components [108–110]. Beyond their initial applicative purpose in food industry [106], novel scopes have been envisaged for these films, especially in the medical field. Egg white-based films treated with chemical crosslinkers, like 1-ethyl-3-3-dimethyl aminopropyl carbodiimide hydrochloride (EDC), for enhanced biodegradation and mechanical properties, have been designed as the base material for fabrication of wound dressing and skin care products. These films showed no sign of cytotoxicity, facilitated the attachment of human dermal fibroblasts and allowed the cells to spread and form filopodia [111]. Blending of silk-fibroin and egg white at various ratios produces composite films, in which the silk fibroin fraction augmented the breaking strength of the films and egg white component contributed in increasing the elasticity and water absorption of the composite, resulting in the adhesion of endothelial cells and their long-term proliferation [112].

Different chemical crosslinkers can be used to fabricated egg white films; however, those that are more biocompatible and induce enhanced biophysical properties are favorable. Primary amine-based molecules like diethylenetriamine (DETA) can polymerize the egg white protein chains and create what is called poly-albumen polymer (Fig. 5b). Egg white films fabricated using this approach show good thermal stability, and enhanced strength and toughness. Moreover, similar to human bone tissues, this poly-albumen films exhibit linear increase in stiffness with time. *In vitro* studies using breast cancer cell lines confirmed that poly-albumen films do not affect the cell proliferation. This construct can be harnessed for different biomedical applications such as implantable electronics [113]. By adding hydroxyapatite crystals to the polymeric matrices prepared using this approach, composite scaffolds can be fabricated and used for bone tissue engineering purposes [114].

Fibers

Egg white can be modeled into fiber-shaped structures. For instance, blend fibers are produced by sulphuric acid-induced gelation of egg white proteins and cellulose fiber formation at the same time [115]. In this protocol, the addition of egg white resulted in rougher fiber surface as well as increased tendency to form β-sheet-type structures and micro-sized fibers. Among the various procedures to manufacture composite fibers from the micro- to nano-scale (1 μm to 100 nm diameter), electrospinning is an advanced technique allowing for tailoring important properties, such as the porosity degree, macropore' size, interconnectivity and interphase tension. Thus far, a plethora of biopolymers have been already shaped into electrospun fibers [116,117]. However, the poor molecular entanglement, caused by the globular molecular structure of pure egg white proteins, reduces their electrospinnability and impedes fiber development [118]. The incorporation of synthetic polymers, such as PVA and polyethyleneoxide (PEO), improves performance and results in homogenous composite fibers [118–120]. Addition of the egg white proteins into PEO solutions before electrospinning greatly influenced the polymer thermal behavior, decreasing its melting point and crystallite formation, while keeping the homogeneity of PEO/egg white fiber diameter (Fig. 5c) [121]. These composite fibers exhibit faster water absorption compared to PEO alone and enable fine-tuning of the fiber diameter.

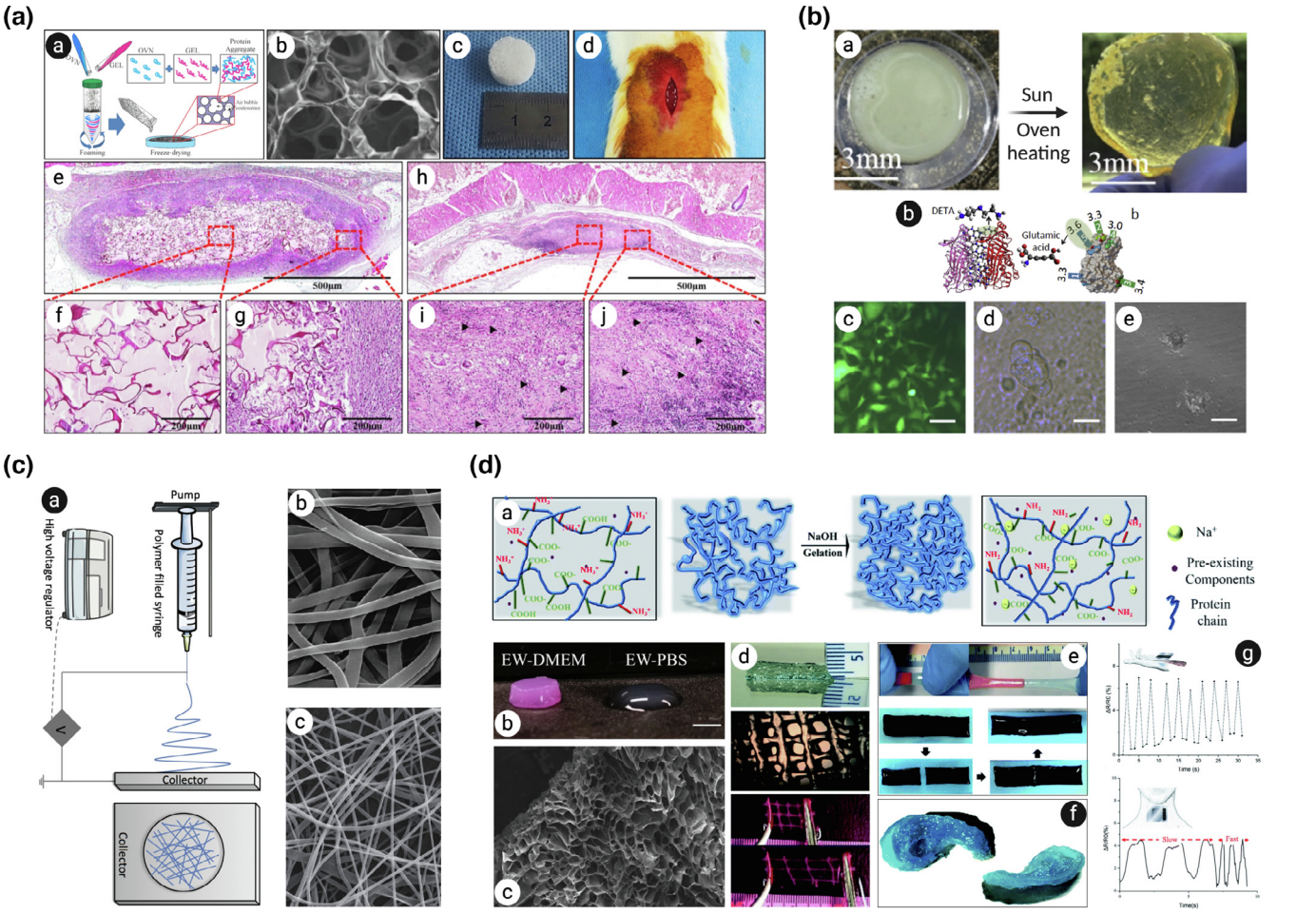

FIGURE 5

(a) Porous ovomucin-gelatin scaffold preparation steps (i, iii) prior to implantation into subcutaneous pocket on rats (iv). H&E stained tissue sections 2 weeks (v–viig) and 4 weeks (viii–x) after subcutaneous implantation. Arrows indicate blood vessel formations (images adapted with permission from: Ref. [101].) (b) Egg white was extracted from an avian egg and a crosslinker (DETA) was added to polymerize it resulting in a flexible and tough material (i, ii). Phase contrast and fluorescent images of the breast cancer cells in 2D monolayer culture after 3 weeks (iii, iv) and after one week of culture on crosslinked egg white film (Scale bar: 20 μm). (Images adapted with permission from: Ref. [113].) (c) Schematic diagram of electrospinning process for nanofiber formation (i). The morphology of the core–shell structured PCL/PEG/lysozyme (ii) and PEO/egg white (iii) composite nanofibers prepared via electrospinning (images adapted with permission from: Refs. [117,118,122]. (d) Schematic illustration of egg white hydrogel formation (i). Alkaline-based egg white hydrogel retains its shape after cell culture medium (DMEM) and phosphate-buffered saline (PBS) soaking (i–iii). 3D printed multi-layer (iv, top) and single layer (iv, bottom) egg white hydrogel mesh retains the original shape after axial elongation in DMEM solution. Egg white hydrogel self-healing (v, top left) and stretchability (v, top left) features. Egg white hydrogels could serve as a matrix for carbon nanotubes (CNT) incorporation without compromising the self-healing properties (v, below). The complex ear-like structure 3D printed using egg white hydrogels (vi). Resistance change response to metacarpophalangeal flexion of the index finger (vii, top) and respiration rate (vii, bottom). (Images adapted with permission from: Ref. [10].)

Besides being used as basal material for fabrication of electrospun fibers, egg white proteins sometimes are also loaded onto electrospun fibers of different natures for controlled release studies or microbiocidal purposes. For instance, lysozyme, as a water-soluble bioactive reagent can form non-woven, biodegradable nanomeshes in combination with Poly(e-caprolactone) as shell and Poly(ethylene glycol) as core (Fig. 5c) [122]. When lysozyme is immobilized onto electrospun chitosan nanofibers, it retained its initial antibacterial activity for longer time and through repeated application cycles, as compared to free lysozyme, demonstrating potential for enhanced and continuous bactericidal use [123]. Although, lysozyme itself is capable of forming fibrils of amyloid type, which further assemble into bigger nanofibers [124,125]. In this process, they arrange in planar orientation in a direction perpendicular to the main fiber axis, while beta-strands build the hydrogen bonds to neighboring molecules [124,125].

Due to the increasing attention towards nanofiber-based materials in medicine and soft matter nanotechnology, novel and faster methods to produce and manipulate fibrils are currently under investigation [126]. Through wet-spinning process and in presence of a polyanionic polysaccharide as cross-linker, lysozyme amyloid fibers are processed into macroscopic fibers. These fibers are capable of releasing small molecules in response to pH variations and mimicking the fibrolamellar structure of bone tissue through oriented calcium phosphate mineralization [127]. Furthermore, the combination of lysozyme fibrils with magnetic materials like nanoparticles (NPs) and their ordering

into a liquid crystal phase under external magnetic fields are also of scientific interest in optoelectronics, photonics and biosensing areas [128–130].

Hydrogels

Thermal treatment above protein denaturation temperature leads to proteins' structure unfolding, aggregation, and exposure of their functional groups (non-polar and sulfhydryl-containing amino acids). The consequent establishment of hydrophobic and disulfide interactions results in a 3D network formation, eventually producing a strong viscoelastic hydrogel [131]. Depending on the salt concentration and pH, two gelation mechanisms are observed in most of the globular proteins, forming either stranded and transparent gels, or opaque and particulate gels [132,133]. Also, through thermal gelation, the egg white could form a hydrogel, whose microstructure differentially affected the swelling behavior [134]. In addition, by adding alkaline solutions (*e.g.* NaOH) at optimal concentrations, the egg white solution forms a solid hydrogel with uniform porous network structures in about 5 min [10]. The resulted egg white hydrogel exhibits autonomous self-healing properties without external stimulation due to non-covalent hydrogen bonding interactions within the hydrogel (Fig. 5d). Since the formation of alkaline-based hydrogels is based on physical crosslinking due to electrostatic repulsion and intramolecular hydrogen bonding, injectable and shear-thinning hydrogels (desirable for 3D printing technology) can be obtained. Egg white hydrogels could be printed into complex human tissue structures (*e.g.* ear). Multilayer printed egg white hydrogels are elastic and recover to their original length quickly after removing the applied force [10].

Moreover, the introduction of certain additives, such as polysaccharides, modulates the hydrogel functional and structural features, and its degradation kinetics. The characteristics of these biomaterials strongly depend on the nature of the biopolymers, the protein-to-polysaccharide ratio, and the environmental conditions (*e.g.* pH and ionic strength). For instance, the addition of glucomannan, gellan gum, and soy isolates allowed for such control on egg white-hydrogel properties [135–137]. A more open structure with wider external surface area is typical of hydrogels with augmented porosity, which can be obtained by various methods, such as solvent-casting, freeze-drying, gas foaming, phase separation, porogen leaching [138], as well as inclusion of porogen agents. In fact, porogen compounds at solid, liquid, and gas phases could be used as templates to create the porous architecture [139]. The addition of gelatin, at various concentrations during egg white heat-coagulation and its subsequent depletion via leaching into 40 °C water yielded to porous hydrogels with different swelling degrees, water-holding capacity, *in vitro* gastric degradation, and textural properties [140]. Bioactive hydrogels could not only be fabricated from whole egg white, but also from its single components. For instance, the gelation of lysozyme at low and high pH results in hydrogels with fibrillar morphology and particulate texture respectively, able to promote cell attachment, spreading and proliferation [141,142]. The amyloid fibrils extracted by lysozyme fragmentation can be lyophilized, stored and then used to produce injectable hydrogels on demand [143].

Hydrogels are ideal systems for creating moist wound dressings to regenerate and repair dermal and epidermal tissues. Egg white hydrogels can be enriched with nanosized particles to create nanocomposites with enhanced properties for some biomedical applications. Adding chemicals or minerals can further boost the physical, mechanical and biological activity of the egg white-based composites. For instance, addition of Na-montmorillonite (MMT) clay to egg white/PVA matrix works as reinforcing agent, improves thermal stability, and emulates the water content and vapor exchange rates of human skin [144,145]. Given the presence of egg white as a source of proteins and the creation of a moister environment, this nanocomposite elicited faster migration of dermal cells and enhanced the collagen formation *in vivo*, as compared to conventional wound dressings [144]. PVA/MMT/egg white nanocomposites with gel content ranging from 79% to 85% and elastic moduli higher than 3 MPa demonstrated to be suitable for practical wound dressing applications [146]. Another example is *in situ* incorporation of gold nanoclusters in luminescent hydrogel matrices derived from egg white. Such platforms could be harnessed for bioprinting, 3D cell culture and diagnostic applications [147].

Particles

Studies on egg white protein–protein aggregation under pulsed electric fields (PEFs) and heat revealed that the dominant binding forces during aggregation are disulfide and weakly noncovalent bonds, respectively [148]. Thermal aggregation of egg white components allows for the production of particles helpful in different applicative areas, ranging from food technology to biochemistry. When OVA aggregates and shapes into larger particles, its allergenicity decreases, making it an interesting case in nutrition science [149]. In biomedical science, egg white/sodium alginate NPs prepared by electronic spray method and loaded with paclitaxel efficiently inhibited colorectal cancer cells *in vitro* [150]. As tested in simulated gastric and intestinal fluids, ovomucin NPs can function as effective pharmaceutical carriers enabling the encapsulation of drugs and their sustained release in intestinal mucosal tissues thanks to favorable mucoadhesive properties and loading profile of both positively and negatively charged substances [151]. More importantly, adding egg white proteins to particle-platforms of different nature enhances the properties and functions of these proteins [152]. The OVA peptide, known to be involved in immune response and investigated for cancer immunotherapy, inhibited the tumor progression more efficiently when loaded onto alginate particles, which served as both carriers and adjuvants [153]. Similarly, the immobilization of lysozyme onto magnetic NPs was found to augment the enzyme stability, with optimal biocatalytic performance with expected utility in the food industry (*e.g.* for winemaking) [154].

Nanogels

With a low density and open network structure, the egg white hydrogels are interesting systems for drug loading and delivery. To this purpose, hydrogels can be generated in nano or micro size in order to promptly react to the environmental stimulations [155,156]. Either physically synthesized or prepared by chemical cross-linking reactions, nanogels combine features of particles and hydrogels in one single nanoplatform [157]. Because of its

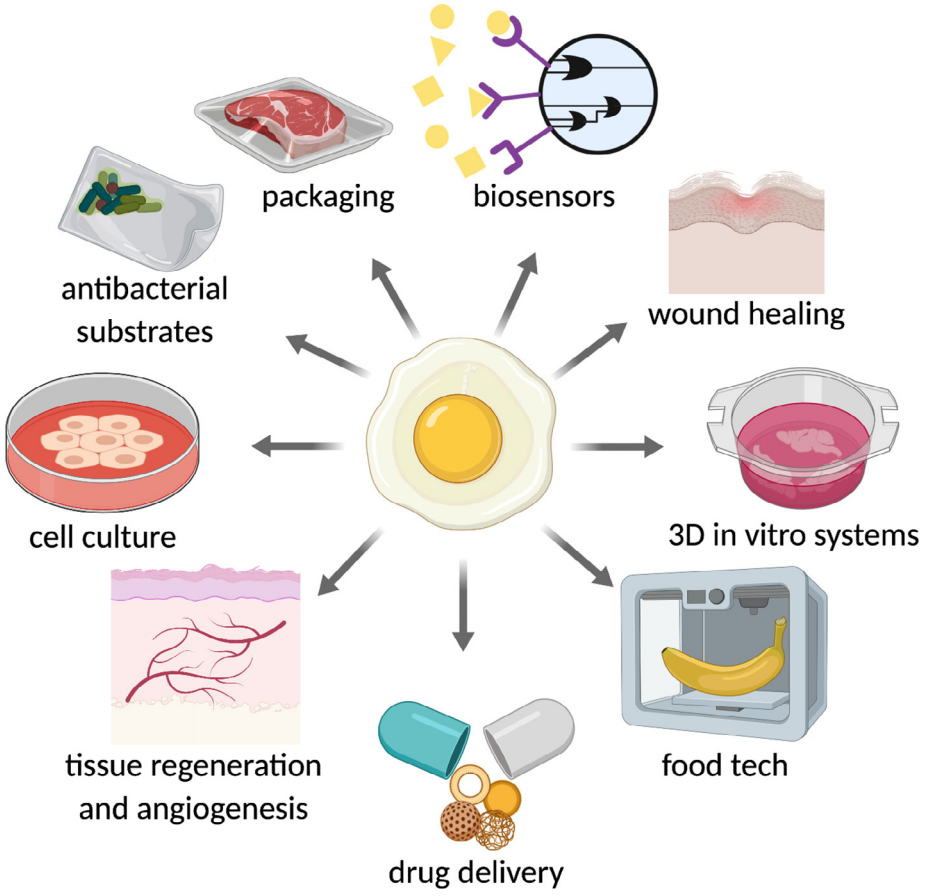

FIGURE 6

Schematic illustration of diverse application of egg white in many fields. Once prepared in a proper shape with required physico-chemical and biological properties, egg white could serve in different technologies (created with Biorender.com).

negative charges, the OVA prevents protein coagulation and stabilizes aqueous colloidal suspensions. These properties have been exploited for manufacturing stable nanogel formulations in combination with other egg white components (such as lysozyme) via thermally-induced protein denaturation and subsequent gelation. In such approach, lysozyme tended to distribute within the internal gel core, whereas OVA remained at the external surface, generating a spherical core–shell architecture with hydrodynamic radius of about 100 nm [158]. By mixing OVA with conalbumin, different, yet stable, spherical nanogels with amphoteric nature were manufactured. A drug model, the benzoic acid, unable to bound to the single proteins in their native states, could be instead loaded in the nanogels by exploiting hydrophobic and electrostatic interactions [159].

Nanogel deformability affects the drug loading and target adhesion affinity in mixed nanogel formulations, like lysozyme-dextran. By introducing various homo-bi-functional cross-linkers, the Young's moduli of nanogels can be tuned, regulating their vascular targeting behavior [160,161]. The overall mechanical properties of the nanogels varied in relation to the structure and hydrophilicity of the crosslinkers, affecting the ability to bind endothelial markers via antibody-mediated recognition [161]. Lysozyme nanogels, in combination with chitosan and amorphous calcium, have found extended applications as dental materials. These heterogeneously sized (50–500 nm) nanogels penetrate the dentinal tubules and enhance dentinal remineralization, thus functioning as mineralized occluding substance by virtue of their dentin-like nature [162]. Finally, lipid droplet-encapsulating egg white microgels generated via injection-gelation process, demonstrated low digestibility rate in simulated gastro-intestinal tract as compared to free lipid droplets, that makes them a good candidate for encapsulation and sustained release of hydrophobic compounds [163].

Biomedical applications of egg white-based biomaterials

Shapeable in a variety of different morphologies, egg white emerged as an extremely attractive material for easy manipulation and use in several fields of human life. Due to their bioactivity, availability and easy manipulation, egg white proteins have found application in diverse areas of biomedicine, including cell culture, wound healing, tissue engineering, 3D cell culture models, pharmaceutics, nutrition and food technology, and biosensing (Fig. 6). Below, we provide an overview of both well-established and innovative applications of various egg white biomaterials thus far described. *In vivo* studies on egg white-based materials for biomedical applications have been summarized in Table 4.

TABLE 4

***In vivo* studies on egg white-based materials for biomedical applications.**

Application	Shape	Formulation	Manufacturing method	Administration route	Dosage	Species	Model	Effects	Study control	Refs
Wound healing	Hydrogel	– Egg white (30 wt.%) – PVA (60 wt. %) – MMT nan-oclay (10 wt. %)	Cyclic freeze/thaw	Topical	N/A	Mouse	Skin wound	Faster healing process; Reduced scar formation; Improved closing of the wound edges; Easier creation of moist wound surfaces; Enhanced tensile strength and elongation-at-break of the healed wound	Sterile gauze	[144]
	– Hydrogel film – Topical hydrogel	N/A	N/A		N/A			Faster migration of epidermal cells; Faster collagen deposition;		[144]
	Ointment	Peptide DG-10	Cyclic freeze/thaw		N/A	Rat		Efficient wound closure	Untreated-Vehicle ointment	[177]
		Egg white	HPLC-fractioning of Ostrich EWH		N/A	Human		Faster wound healing; Reduced necrosis; Decreased exudate secretion; Reduced wound induration, peripheral edema, granulation, and epithelialization tissue	Placebo-silver sulfadiazine cream	[176]
Pharmaceuticals	Emulsion of egg white lysozyme	– Lysozyme (50 μg) – Complete Freund's adjuvant	N/A	Subcutaneous	N/A	Mouse	Inducible nitric oxide synthase (iNOS) knockout	Reduced blood flow in venous microcirculation	Saline	[229]
	Solution of egg white peptides	YRGGLEPINF and ESIINF peptides	N/A	Oral	10 mg/kg BW	Rat	Spontaneous hypertension	Vasodilator and immediate antihypertensive effects	Saline	[230]
		Ile-Arg-Trp (IRW) peptide	N/A		3 and 15 mg/kg BW			Blood pressure reduction; Restoration of circadian variations in blood pressure; Restoration of NO dependent vasorelaxation; Decreased Angiotensin II levels and increased circulating bradykinin (inhibition of ACE-I); Ameliorated oxidative/nitrosative stress and fibrosis; Ameliorated inflammation	Vehicle	[231]
			Fmoc solid-phase synthesis	Intrasystemic	15 mg/kg BW			Increased ACE-2 expression and decreased proinflammatory genes expression in mesenteric artery	Saline	[232]

(continued on next page)

TABLE 4 (*CONTINUED*)

Application	Shape	Formulation	Manufacturing method	Administration route	Dosage	Species	Model	Effects	Study control	Refs
		IRW peptide TNGIIR and RVPSL peptides	N/A Enzymatic digestion of OVA HPLC purification	Oral Intrasystemic	15 mg/kg BW 2, 10 and 50 mg/kg BW			Upregulated ACE-2 protein levels in kidney and aorta Anxiolytic activity Blood pressure reduction; Enhanced endothelium-dependent vasorelaxation; Decreased vascular inflammation	Vehicle – Saline – Captopril – Saline – Mas receptor antagonist A779	[233] [234] [192]
	Solution of lysozyme	Lysozyme	Enzymatic digestion of OVT	Oral	100 mg/kg BW	Mouse	Mammary carcinoma	Reduction of lung metastasis and number of the lymphatic nodules under the layer of epithelial cells in villi; Recovery of the response of mononuclear cells	Vehicle (control)	[235]
	Solution of OvoM	– OvoM (0.1%) – Ofloxacin (OFLX, 0.3%)	N/A	Ocular	N/A	Rabbit	P. aeruginosa keratitis	Reduced corneal damage	– Saline – OFLX – OvoM	[236]
	Solution of egg white-derivatives	Egg white-derivatives	Separation from egg white	Oral	400 mg/kg BW	Cat	Cyclophosphamide (CPA)-induced immunosuppression	Increased numbers of platelets, white blood cells (WBC) and neutrophils; Enhancement in the phagocytic activity of neutrophils	Saline	[237]
	Solution for OVT	OVT	Microimmunoelectrophoresis purification		50 and 250 mg/kg BW	Mouse	Dextran sodium sulfate (DSS)-induced colitis	Reduced clinical signs, shortening of the colon, and weight loss; Reduced inflammatory cytokine markers	– Water – DSS	[238]
	Solution of mannosylated egg white	Mannosylated egg white	N/A		1 mg/dose		Allergy	Reduced clinical signs, serum histamine, mouse mast cell protease (MMCP); Reduced activity of antibodies (IgG, G1, and E) and cytokines (IL-4); Increased IL-10 and T regulatory cells	– Cholera toxins-Glucosylated peanut – Mannosylated peanut, and whey	[239]
	Solution for egg white OVT (100 μL)	– Apo-OVT or Holo-OVT (1 mg) – Cholera toxin B subunit (10 μg)	HPLC purification		1 mg/dose			No systematic allergic signs; Lower serum levels of mouse mast cell protease-1 and IgE; Lower levels of IgG1, as well as the Th-1 IFNγ and Th2-type IL-13; Reduced dendritic cell uptake	– Distilled water – Apo-OVT	[240]

TABLE 4 (*CONTINUED*)

Application	Shape	Formulation	Manufacturing method	Administration route	Dosage	Species	Model	Effects	Study control	Refs
Nutrition	Egg white powder	AIN 76A diet + egg white powder (9% w/w)	Purification and Maillard reaction	Oral	N/A	Mouse	Aging	Increased serum sulfur AA, cardiac GSH and glutamine transporter ASCT2; Reduced cardiac cysteine carrying transporter SNAT2; Increased IL-10 expression; Potent anti-oxidant and mild anti-inflammatory effects	AIN 76A diet + casein (9% w/w)	[150]
	Egg white gels (8.7% proteins)	– Granular-spongy (pH 5; IS 1 M) – Intermediate (pH 7; IS 1 M) – Smooth-rigid (pH 9; IS 0.05 M)	Electrophoresis Fractioning and iron chelation		N/A	Pig	Digestion	Modulating gastric pH	N/A	[241]
	EWH	High fat diet (HFD) + 4% EWH	Drying and powder production		N/A	Rat	Metabolic Syndrome	Increased glucose tolerance and insulin sensitivity in adipose tissue and skeletal muscle; Reduced systemic inflammation; Increased adipocyte differentiation	HFD + variable % EWH	[242]
	Solution of Lactic-fermented egg white	Drink containing lactic-fermented egg white	Heat-induced gelation		N/A	Human	Obesity	Tune visceral to subcutaneous fat area ratio; Reduced visceral fat area	Whey-based drink	[243]
	Egg white	Diet with egg whites or yolks in presence of glucose	Enzymatic digestion		N/A		Pre-diabetes	Attenuated postprandial hyperglycemia-induced oxidative stress; Reduced lipid peroxidation; Vasoprotection; Improved arginine metabolism	Glucose	[244]
	Diet with egg white in place of fish/meat	Egg white	Fermentation with lactobacillus		N/A		Hyperphosphatemia in dialysis	Decreasing the serum phosphate; Maintaining the body weight and serum albumin concentration	Unvaried diet	[245]
Tissue engineering	Macroporous sponge	Egg white	Lyophilization	Subcutaneous	Soft/vascular tissue engineering	Mouse	Subcutaneous implantation	Low immune reaction; Enhanced angiogenesis; Effective tissue ingrowth	Collagen sponges	[28]
						Rat		Aberrantly enlarged vascular structures;	Collagen sponges	[96]

(continued on next page)

TABLE 4 (*CONTINUED*)

Application	Shape	Formulation	Manufacturing method	Administration route	Dosage	Species Model	Effects	Study control	Refs
	Porous scaffold	– Ovomucin (50%) – Gelatin (50%)	EDC crosslinking		Bone or dermis tissue engineering		Increased vascularization over time; Limited fibrosis; Minimal allergic responses; Activating macrophages favorable for tissue repair	N/A	[101]

BW: Body weight.
OVT: Ovotransferrin.
EWH: Egg white hydrolysates.
OvoM: Ovomacroglobulin.
N/A: Not available.

In vitro cell culture models

Egg white proteins affect cell behavior in different ways. These proteins better preserve osmolality and viability in human skin fibroblast cultures with respect to human saliva [91], in good accordance with previous discovery that lysozyme can stimulate both their anchorage to substrate and spreading after trypsinization [164]. Ovomacroglobulin promotes fibroblast migration by enhancing cell adhesion to extracellular matrix, strengthening the cytoskeleton and reducing intercellular aggregation [165]. OVA and OM promote myoblast cell proliferation, whereas conalbumin inhibits it and lysozyme has no effect [166]. Some of these protein fractions separated by tandem ion exchange chromatography possess proliferative bioactivity in cells of different origin [167]. For example, egg white components smaller than three kDa stimulate cell proliferation and survival in 293T cell line, derived from human embryonic kidney cells [168]. Moreover, egg white maintains the cell survival and sustains the differentiation of spleen cells [169]. Given the positive effects on cell behaviors, egg white hydrolysates have been therefore used to enrich the cell culture medium of multiple cell lines for years [170].

Due to a number of favorable features, such as temperature-tunable viscosity, optical transparency to monitor cells, availability and low cost, egg white has emerged in the search for reliable and economical substitute supplements for commercial 3D cell culture medium. For instance, certain egg white-based culture supplements improved 3D organotypic models of immortalized human breast epithelial cells (MCF10A) [171], salivary gland cells [172] and endothelial and smooth muscle cells from human and rodent origin [173]. Similar to reconstituted basement membrane preparations such as Matrigel, MCF10A cells cultured in egg white forms organized acinar structures with apico-basal polarity (Fig. 7a). However, the 3D cell growth in egg white is not tissue or species restricted. Other established cell lines from different tissues, such as breast cancer (MCF7), human embryonic kidney (HEK293), human cervical cancer (HeLa), human prostate adenocarcinoma (LNCaP) and human osteosarcoma (Saos-2) cell lines, show comparable morphology in both egg white and Matrigel culture systems [171].

Wound healing, tissue regeneration and angiogenesis

The presence of proteins with cell-stimulatory properties and the abundance of antimicrobial compounds make the egg white an interesting material in designing and optimizing biological engineering strategies for wound healing and tissue engineering. The treatment of chronic wounds often combines wound healing, antibiotic therapy, and sometimes surgical removal of damaged tissues. When topically applied, the tryptophan, a vital substance and essential amino acid found in egg white, augmented the re-epithelialization, cell proliferation and neovascularization, helping the healing process of murine and human burn skin wounds [174,175]. Egg white-based ointment improved recovery rate in second-degree chronic burn wounds in humans, where wound depth, necrotic tissue, exudate discharge, peripheral edema and granulation were considerably decreased versus the egg white-free dressings [176]. Furthermore, one of the potent antioxidant peptides identified in egg white hydrolysates, called DG-10, exhibited promising wound-healing activity closing more than

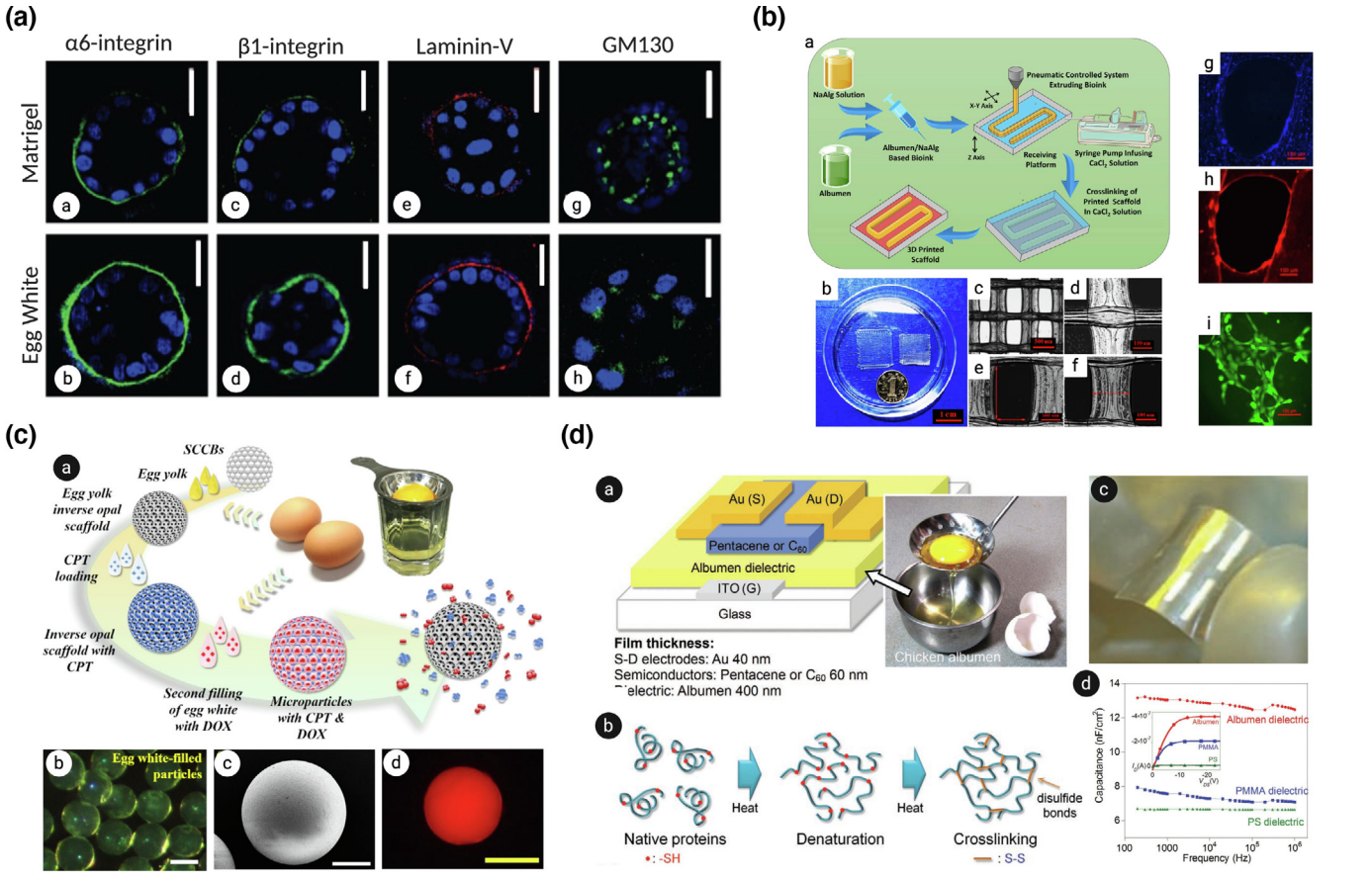

FIGURE 7

(a) Apicobasal polarization of MCF10A cells cultured in egg white and Matrigel for 12 days and stained for α6-integrin (i, ii), β1-integrin (iii, iv), laminin-V (v, vi), and GM130 (vii, viii). Nuclei were counterstained in blue. Scale bar: 25 μm. (Images adapted with permission from: Ref. [171].) (b) The schematic illustration of egg white/sodium alginate (NaAlg) composite scaffold preparation process (i). The printed egg white/NaAlg composite scaffolds (ii) with grid (iii), perpendicular stacked (iv), the pore (v) and the filament (vi) structures. Human endothelial cells stained with DAPI (blue) and Phalloidin (red) and Live/Dead (green) showing scaffold structural boundaries (vii, viii), and endothelial sprouting and vascular networks within the egg white/NaAlg composite scaffolds after 4 days of culture. (Images adapted with permission from: Ref. [12].) (c) Schematic diagram of the fabrication and synergistic drug delivery application of the egg-derived inverse opal microparticles (i). Reflection images of the egg white hydrogel-filled inverse opal microparticles (ii, Scale bar: 200 μm). Whole view of the egg white filled inverse opal particle (iii). Scale bar: 100 μm. The hydrophilic drug (DOX) could be loaded into the microparticles during the egg white filling process (iv). Scale bar: 200 μm. (Images adapted with permission from: Ref. [193].) (d) The schematics showing denaturation of egg white protein and the crosslinking reaction under the thermal treatments during fabrication of OFET using egg white dielectrics (i, ii). A photograph of the device configuration (iii). The capacitances of egg white, PMMA, and PS, corresponding to driving frequencies of 200 Hz to 1 MHz at 25 V (iv). (Images adapted with permission from: Ref. [220].)

90% of 1 cm-wide full thickness wounds in adult rats [177]. Thanks to these remarkable wound healing properties, number of film and hydrogel platforms have been fabricated from egg white to dress acute and chronic wounds [111,112,145].

Not in wound healing alone, but also in tissue engineering facets, egg white is harnessed as a supporting material to constitute scaffolds for tissue regeneration and vascular ingrowth [28,101,112,178]. Various egg white-derived formulations satisfy some of the contingent criteria for implantation into living tissue, such as tunable physical characteristics. As mentioned above, angiogenesis plays a crucial role in cell survival and integration of the newly formed tissue into the host. For this reason, egg white constructs find extensive application in tissue engineering. In fact, their capability to interact with growth factors, the density and architecture of their internal matrix, and other features sustain and strongly prompt the vessel ingrowth [28]. Similar to Matrigel and collagen matrices, egg white could be also used as an ECM substitute for *in vitro* 3D angiogenesis assays, where the formation of the vascular tubule networks can be easily observed upon fluorescent labeling of cells [173]

The combination of a polymeric matrix with solid particles is a common strategy to fabricate scaffolds for hard tissue regeneration. Egg white has previously been incorporated in bone tissue engineering multi-layered apatite-based constructs, where polymerized egg white acted as a load transfer entity to induce toughness to hydroxyapatite crystals [114]. At the interface between the egg white and the crystals, hydrogen and ionic bonds are established which contribute to the high toughness and stiffness of the scaffold, respectively. In another report, egg white ovalbumin was combined to a synthetic polymer (poly-methyl methacrylate, PMMA) and a solid phase composed of Ce–Cu substituted apatite to manufacture a novel bone scaffold with enhanced antibacterial property, cytocompatibility and degradation rates [178].

3D printing, as one of the strategies currently used in tissue engineering, is capable of generating anatomical structures with highly tunable compositions, reproducible architectures and patient-specific requirements. In this system, bioinks provide temporary environment for the cells to attach, proliferate and differentiate into tissue-specific lineages. To develop a new product with high printability and biocompatibility, overcoming the limitations of natural-based bioinks, a composite bioink consisting of sodium alginate (NaAlg) and egg white was developed. *In vitro* experiments indicated that human umbilical vein endothelial cells cultured on a bioprinted NaAlg/egg white scaffold can maintain high viability and develop vascular sprouting and neovascular networks in between fibers of printed scaffold [12]. This composite 3D printed scaffold has great potential in tissue/organ engineering and induction of vascularization within the bioengineered tissues (Fig. 7b).

Pharmaceuticals

Egg white-derived bioactive peptides are also used in the pharmaceutical industry, either in their native state or after modification with enzymes. In this area, the main functions are: (*i*) transportation of drugs and metal chelation, (*ii*) antimicrobial activity, (*iii*) anticancer activity, (*iv*) antioxidants, (*v*) antiviral, (*vi*) immunemodulatory, and (*vii*) antihypertensive activities [35,76]. Below we introduce the potential of a few egg white-derived substances towards pharmaceuticals applications. Entire cell organelles extracted from the egg white, like the lysosomes, displayed prolonged antimicrobial activity towards E. coli with neither bacterial resistance nor cytotoxicity against mammalian cells [84]. Increasing the permeability of the bacterial outer membrane by forming large size pores, lysozyme displays well-recognized bactericide properties especially against Gram-positive bacteria [179]. Self-assembled mixed β-lactoglobulin–lysozyme microspheres were developed as bioactive vehicles in the formulation of food products and pharmaceuticals (*i.e.* nutraceuticals) [180]. Conalbumin, as a well-recognized iron binder and transporter, could serve as an iron supplementing agent. Moreover, being a superoxide dismutase (SOD)-mimicking protein with a potent superoxide anion (O^{2-})-scavenging activity, it also exhibits notable O^{2-} dismutation capacity [181]. OVA demonstrates substantially improved antioxidant activity after conjugation with polysaccharides [182], selenite [183] and buckwheat polyphenol rutin [184]. Ovomucin exhibits antibacterial, antiviral, and antitumor activities, and suppresses cholesterol absorption, while its derived peptides present metal chelating, antioxidant and angiotensin-converting enzyme inhibitory actions [185–187]. Recently, intensive research has been carried out to produce and characterize enzymatic hydrolysates of OVA, ovomucoid, and ovomucin [53,187–189]. The regulation of immune responses triggered by egg white hydrolysates has been studied in human peripheral blood cells [190]. Besides reducing the synthesis of angiotensin II, endothelial cell inflammation and endothelial dysfunction, a conalbumin-derived tripeptide was found to attenuate the angiotensin-induced over-proliferation, superoxide production, and inflammation in vascular smooth muscular cells [191], reducing blood pressure in *in vivo* models of hypertension [192].

Apart from individual egg white proteins, whole egg white could be incorporated in formulations of drug delivery constructs. Egg white has previously been used in microparticle-based drug delivery systems, which are highly controllable, capable of loading sufficient amounts of drugs and allow monitoring the process of release. In this dual filling delivery system, the egg yolk inverse opal particles were initially fabricated by the negative template replication of silica colloidal crystal beads, and then an egg white pre-gel solution was added to achieve the secondary filling of the hydrogel into the inverse opal particles. Different hydrophobic (*e.g.* camptothecin) and hydrophilic (*e.g.* doxorubicin) drugs were encapsulated into these microparticles at different stages and enable a synergistic drug delivery (Fig. 7c). The sustained release of the two drugs significantly reduced the viability of human liver cancer cell lines (HepG2) [193]. Nevertheless, the actual clinical applicability of this egg-based delivery system in the treatment of tumors remains to be demonstrated.

Nutrition technologies

As a source of high quality proteins and functional lipids as well as valuable minerals, carbohydrates, and vitamins, the egg is one of the most versatile foods, providing complete nutrient bioavailability [179]. In the form of dried powder (advantageous in relation to storage, microbiological safety, and transportation), eggs are extensively used in the food industry for the preparation of bakery and convenience food, sauces and confectionaries [194]. Specifically, the egg white components are of particular interest for their antimicrobial and antioxidant activity, protease function, storage stability, and reduction of protein allergenicity by enzymatic hydrolysis [35,195–197]. Importantly, as functional food ingredient with high water solubility, digestibility, absorption in gastrointestinal tract, and defined hypolipidemic properties, the egg white hydrolysates have prospects for their inclusion into dietary products for prevention and treatment of the metabolic syndrome [198]. The combination of egg white with other materials has proven effective in enhancing the properties of edible egg white-based materials [199]. For instance, the addition of polysaccharides (*e.g.* galactomannan) enhances the antioxidant effect of ovalbumin and the antimicrobial activity of lysozyme [200]. Carrageenans, as another example of polysaccharides family, improve characteristics, such as mechanical stability, consistency, texture and taste [201].

In most foods with foaming characteristics, proteins stabilize the dispersed gaseous phase. Due to its excellent aeration capacity, the egg white protein is used as a surface-active ingredient for aerated confectionery. Pectin, a carboxylated anionic polysaccharide, serves instead as food gelling and thickening agent [202]. Studies have shown that the electrostatic interactions between pectin and egg white proteins increase the foam stability. This polysaccharide provides the egg white foams with electrical neutrality and a viscoelastic interfacial network at the air–water interface, which reduces the gas permeability and enhances the resistance to disproportionation (air diffusion from a small bubble to a big bubble or to the atmosphere) [203].

Food packaging

Food safety is an important topic affecting public health. Active and smart packaging are recently gaining strong attention as

they enable real-time monitoring of the food quality [204]. Increasing efforts have been dedicated to incorporate multifunctional sensing systems to manufacture smart packaging biomaterials with tunable or responsive properties that can monitor gas production, humidity, temperature and growth of microorganisms [205–207]. Food packaging materials can be produced by mixing biopolymer formulations with plasticizers and disrupting agents (like water or glycerol), which, by weakening the intermolecular forces, increase the mobility of the polymeric chains, lower the material glass transition temperature, and reduce its brittleness [208]. Massively produced, soy and egg white proteins are appealing renewable candidates for food packaging preparations [209,210]. Multiple reports have, in fact, already demonstrated the feasibility of production of egg white-plastics by thermo-mechanical methods [210–212]. Subsequent characterization of thermal, optical, mechanical, and moisture absorption properties revealed that these materials are good candidates for applications in food packaging and material molding processes [83,210]. However, to further enhance the physical properties, other additives, such as polysaccharides (*e.g.* tragacanth gum, cellulose and alginate), are often added to increase the water uptake or soften the structure by lowering the values of flexural moduli and tensile properties [136,199,213,214]. Edible egg white/cellulose nanofibrous composites have been proposed for textile applications and food packaging [115,215]. In this system, the egg white component increases the storage modulus and surface roughness of the composite fibers, while maintaining the native monoclinic crystalline structure of the cellulose.

In light of the progress achieved in applying the egg white-based products in other biomedical areas (such as sustained drug delivery systems, tissue engineering scaffolds or antimicrobial surfaces), we expect that these biomaterials will experience tremendous growth also in food packaging technology in the future. Moreover, having proper physico-mechanical characteristics on one side and antibacterial features on the other, egg white could serve not only for packaging of food, but also for production of smart drug capsules in pharmaceutical industry [83].

Biosensors and bioelectronics

As natural source biomass working as chelating and reducing agents, egg white has also been involved in the development of biosensing systems, usually with the function of template and stabilizer. When hybridized with targeting food proteins, the gold nanoclusters (AuNCs) are excellent biosensors to detect food contaminants and bioactive nutrients [216]. Through an egg white-assisted one-step green synthetic approach, AuNCs were synthesized to build a fluorometric platform for highly sensitive detection of organophosphorus pesticides and mercury ions [217,218]. Here, the egg white was directly employed without purifying any protein fraction. Furthermore, the egg white proteins exerted reducing and protecting functions in AuNC-based fluorogenic biosensors developed to measure the Cu(II)-induced prooxidant activity of natural antioxidants abundant in biological samples and foods [219]. Egg white in the form of a hydrogel incorporating conductive carbon nanotubes were printed by direct ink writing to manufacture wearable electronic sensors to capture various body motion signals [10].

Given the remarkable flexibility and facile manipulation, the egg white-based hydrogels with incorporation of conductive carbon nanotubes (CNTs) have been 3D printed to generate electronic sensors [10]. These sensors are able to capture and analyze various motion signals in the human body, including vigorous finger bending and delicate motions like wrist reflection of index finger flexion and pulse. Interestingly, discrete activities (like respiration frequencies) can also be monitored in real-time through these composites (Fig. 5d). Combining the attributes of direct 3D printing and high flexibility, 3D printed egg white-CNT sensing hydrogels offer a promising platform for the next generation of wearable electronics and stimulus–responsive actuators.

Thanks to the biodegradability, biocompatibility, and environmentally friendly features, the application of egg white-biomaterials in organic electronics has gained considerable attention in recent years. Bioorganic field-effect transistors employ biomaterials as semiconductors, dielectrics, and substrates to simplify the fabrication process while decreasing the production costs in organic electronics. Egg white has been previously used as a gate dielectric in organic field-effect transistors (Fig. 7d). Interestingly, the output currents of transistors made with egg white dielectrics doubled those generated in common polymeric dielectrics, such as poly(methyl methacrylate) and polystyrene dielectrics. The high dielectric constant of albumen is one of its advantages in organic field-effect transistor applications [220]. As a versatile inexpensive material, easily manipulated through green processing, the egg white easily enters innovative applications such as bio-actuators, wearable devices, and biosensors.

Conclusions and future remarks

Egg white has shown great utility and potential as a biomaterial for various biomedical applications, particularly in the areas of wound healing, tissue engineering, *in vitro* cell culture, drug delivery, nutrition, biosensor and bioplastic technologies. This new old material demonstrates attractive features such as high bioactivity and availability, biocompatibility, easy handling and low production costs. Moreover, since egg white proteins exhibit versatile physical properties, like foaming, gelling and emulsifying activities, they can be shaped into various morphologies such as sponges, hydrogels, films, fibers, nanogels and particles. Where physico-mechanical properties and stability of these constructs are unsatisfying, whole egg white, its proteins or their derivatives are sometimes chemically crosslinked or mixed with other biocompatible synthetic polymers. Egg white-based composites find numerous applications from green synthesis of electrocatalysts [221] to smart sensing hydrogels [10].

Egg white proteins show favorable biological activities, such as antimicrobial, growth factor binding and cell growth and attachment stimulatory properties. In light of these advantageous biological properties, egg white scaffolds have shown great promise in tissue engineering applications, particularly in inducing new blood vessel formation and promoting the host tissue integration. While *in vitro* and *in vivo* studies confirm the inherent VEGF binding potential in egg white sponges and their subsequent angiogenic modulatory response [28,96], future studies

will help us understand the interaction between other growth factors or chemokines with egg white biomaterials. Moreover, the successful application of egg white-based materials in tissue engineering requires a deeper comprehension of the long-term biocompatibility as well as a better ability to precisely tailor their morphologies and adapt their characteristics to tissue specific requirements. Strategies to generate hybrid materials or adding native ECM components to egg white matrices could address the aforementioned concerns.

Although egg white in its native form is known to induce allergic responses in some people [222], no adverse immune reactions have been reported upon implantation of egg white biomaterials in animal species [28,96,101]. That said, further investigations are still required to understand the underlying complex immune responses occurring in animals in order to properly translate these materials towards clinical applications. Egg white biomaterials display efficient engraftment, vascularization and targeted drug delivery, which are critical towards clinical translation. Moreover, they are fabricated via facile, inexpensive approaches and find utilization for myriad of applications, such as wearable devices and biosensors. Although preclinical models can provide important information on biocompatibility and efficacy of these biomaterials, many regulatory hurdles must be overcome in order to demonstrate safety and efficacy in clinical translation. Regulatory certification standards and manufacturing tactics throughout the product development process, as well as the compliance to the current good manufacturing practice (GMP) process are among the important parameters in their clinical applications [223,224]. Batch-to-batch variations, adverse immune responses and allergies, long-term host-tissue receptivity and cell-tissue integration, as well as mutagenic effects are some of the factors that could potentially hamper this translation process. One approach is to study individual egg white proteins in a one-at-a-time approach to potentially target those that might induce unfavorable behaviors. The other is to devise multidisciplinary approaches to bring chemical, physical, biological and manufacturing fields together and introduce *de novo* design techniques to tackle the aforementioned challenges. Lastly, extensive preclinical experiments using advanced *ex vivo* or *in vivo* models that could recapitulate human responses will complete the assessment of the clinical translatability. In light of the recent progress in the field and the growing attention to egg white biomaterials, we envision more translational developments to come in the next decade.

Data availability

The authors declare no data availability.

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None.

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