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An Egg Shell: A Nutritional Profile and Health Benefits

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ABSTRACT

Calcium is one of the most fundamentally significant and essential component for the human body since it makes up the majority of the skeletal system in humans (98%). The goal of the present review study was to discuss calcium's importance in everyday life, calcium absorption theories, eggshell (ES) deposition, and its nutritional makeup. There are two phases to ES formation. 5.5 grams of calcium carbonate, or about 95% of the dry ES, are present. One ES contains traces of sodium, potassium, zinc, manganese, iron, and copper as well as 0.3% phosphorus, 0.3% magnesium, and sodium, potassium, zinc, manganese, and iron. Vitamin D is necessary for calcium absorption; hence, calcium bioavailability is influenced by dietary calcium intake as well as vitamin D status. Calcium may reabsorb at a number of places along the nephron. Intestinal acidity (particularly for CaCO3 absorption), oestrogen, vitamin D, and soluble fibre/prebiotics, probiotics, and synbiotics are some of the elements that have been shown to favourably boost calcium absorption. Pulsed electric fields, high-energy milling, and electric discharge-assisted mechanical milling are relevant techniques that have been studied and might be used to extract calcium from ESs. Researchers looked at the health benefits of eggshells and different ways to get the calcium out of eggshells to see if they could be used to make a good calcium supplement.

KEYWORDS: Egg shell, Nutrient content, Extraction method, Calcium absorption, Patents

INTRODUCTION Given that it makes up 98% of the skeletal system and is one of the most fundamentally necessary and crucial elements for the human body, calcium is obtained through dietary sources (Muñoz-Garach et al., 2020). It performs a crucial structural role in our bodies, so its purpose is obvious. Furthermore, records indicate that India's dietary calcium intake has been declining over the last 50 years (Kadhim et al., 2020). A crucial micronutrient, calcium is required for the development of teeth and bones as well as muscle contraction, blood clotting, and nerve conductivity (Szymandera-Buszka et al., 2021). To prevent hypertension and preeclampsia, it is advised to consume adequate calcium, which is essential for cardiovascular health (Arnold et al., 2021). People are becoming less concerned with consuming adequate calcium as time goes on, which might result in illnesses like osteoporosis and hypocalcemia (Kadhim et al., 2020) and worse bone density. A steady reduction of bone density is a defining feature of osteoporosis (Fayet-

Research has looked for innovative ways to include egg shells (ESs) in the human diet to satisfy its calcium demands since they are a substantial source of calcium. According to several

clinical studies, giving some organisms calcium from ESs led to increased bone mineral density and an anthracitic effect. Osteoporosis is characterised by increased bone density, reduced pain perception, and greater mobility. The high bioavailability of ES calcium has been linked to improvements in asthma, lethargy, brittle fingernails, hair, and other conditions in both children and young people (Bartter *et al.*, 2018; Lorenc *et al.*, 2018; Shafiei *et al.*, 2019; Ahmed *et al.*, 2022). The current study looked at the nutritional value of ES, the importance of calcium in the diet, and the things that are needed to help the body absorb calcium.

Egg Shell (ES)

The primary function of ES is to act as a physical barrier that blocks germs from entering cells while allowing for gas exchange (Jayasree *et al.*, 2018). An unwanted byproduct is chicken ESs. You may substitute them for crustacean shells, and they are a rich source of calcium for the diet (Polo *et al.*, 2018). In ES deposition, there are two steps. In chicken breeds that are highly chosen as layers, the longest stage of egg development lasts for around 17 hours. The initial phase of mineralization begins within the first five hours. The first calcite crystals begin to develop at the sites of the organic aggregates located on the surface of the outer shell membranes. The composition

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Moore et al., 2019).

of these aggregates is unclear, notwithstanding the suggestion that they include a keratan sulphate proteoglycan. The genetic makeup of each species affects the location of these nucleation sites differently (Abo El-Maaty et al., 2021; Lee et al., 2021; McClelland et al., 2021;). The separation between these locations dictates the size of the mammillary cones, the cylindrical diameter of the palisades in the compact layer of the shell, and ultimately the strength of the shell. At the beginning of mineralization, radial calcite crystals form. The nucleation sites serve as the origins of mammillary cones (Zhang et al., 2022). As they slowly converge to form the bases of the palisade layer as they extend outward, radial crystal development is stopped by mutual exclusion. The second step (Li et al., 2021) involves the polycrystalline calcite rapidly growing to form the palisade layer. Ovocalyxin-32, a major phosphoprotein in the ES matrix, is mostly found in the outer ES and cuticle and may be a candidate for a protein crystal growth inhibitor (Zhao et al., 2020; Baláž et al., 2021; Gautron et al., 2021; Younas et al., 2021).

Nutritional Profile of ESs

The chicken ES is made up of shell membrane and calf ES. Its overall weight is 10-11% less than the weight of the whole egg. The chemical makeup of the ES is 98% dry matter and 2% water. On the other hand, ash takes up 93% of the dry matter, whereas crude protein only accounts for 5%. The egg's membrane is a fibrous structure. It is essential for the growth of ES (Chen et al., 2021). Chemically, an ES from a hen has 65.6% water, 11.8% protein, 11% fat, and 11.7% ash. ES powder is chemically composed of 21.2% carbon, 0.93% magnesium oxide, 76.9% calcium oxide, 0.42% porosity, 0.02% iron oxide, and 0.11 percent sodium oxide (Wang et al., 2017; Bello et al., 2020; Ma et al., 2020; Hsieh et al., 2021) (Figure 1). Additionally, 0.3% of ESs are composed of phosphorous, which is scarce but is very helpful for repairing hen bones. Hen ES is also present in magnesium, which contributes to preserving the ES's quality. It is at a 0.2% concentration. The shell contains between 28.2 and 41.2% calcium and 0.102 percent phosphorus (Hsieh et al., 2021).

Theory of Calcium Absorption

Because vitamin D is required for calcium absorption, dietary intake and vitamin D status both affect how much calcium is bioavailable. The efficiency of absorption is dose-dependent and associated with physiological calcium needs (Nassar & Alotaibi, 2020). Conversely, lactose promotes the absorption of calcium. Calcium absorption involves both a paracellular mechanism that is passive and a carrier-dependent process that is active. Although the active process requires vitamin D, the passive process does not. Low calcium intake triggers the conversion of 25(OH)D to 1,25(OH)2D, which boosts the transcription of the gut's calcium transport proteins. However, this homeostatic regulation mechanism is inadequate to make up for continuously low calcium intakes. A reduced conversion to 1,25(OH)2D occurs when vitamin D stores are very low (Cao et al., 2020; Kobus-Cisowska et al., 2020; Mensah et al., 2021; Mulualem et al., 2021). Another strategy is net calcium absorption, which is based on metabolic balance and requires stringent food restrictions as well as extensive urine and faeces collections. This method does not stop the endogenous secretion or the ingested calcium that is re-excreted in the gut. Changes in blood calcium levels or Ipth (parathyroid hormone) after a calcium challenge are indirect signs that calcium is being taken in. The methods by which calcium crosses the intestinal barrier are both saturable (presumably transcellular) and nonsaturable (likely paracellular diffusion) (Wang et al., 2017). The duodenum, the proximal part of the small intestine, and to a lesser degree, the jejunum, have a saturable capacity for calcium absorption (midportion of the small intestine).

Controlled paracellular transport also helps the body absorb calcium. The jejunum and ileum generate larger amounts of 1,25(OH)2D, which improves passive transport by producing tight junction proteins claudin-2 and claudin-12 (Bello *et al.*, 2020). Vitamin D controls active calcium absorption in the proximal duodenum and jejunum, as opposed to the ileum, where claudin-2 and claudin-12 expression is the greatest. Claudin-15 is an enzyme that helps the paracellular calcium absorption process and is upregulated by prolactin during pregnancy and breastfeeding. Transient receptor potential cation channel subfamily V members TRPV5, TRPV6, and

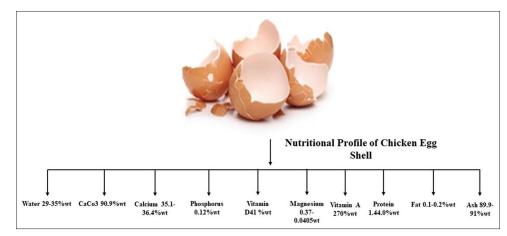


Figure 1: Nutritional values present in ES

calbindin-D9K are also upregulated, as is transcellular calcium transport. The voltage-dependent L-type calcium channel subunit alpha-1D, sometimes referred to as voltage-gated calcium channel subunit alpha Cav1.3 may also help in calcium absorption from the gut. Even though the gene for this protein is not controlled by vitamin D (Kuwataka, 1994; Cao *et al.*, 2020; Kobus-Cisowska *et al.*, 2020; Nassar & Alotaibi, 2020; Hsieh *et al.*, 2021; Mensah *et al.*, 2021; Mulualem *et al.*, 2021), there are no major changes in calcium or bone metabolism in animals that don't have Cav1.3.

It is impossible for a single model to fully account for the timing of reactions to 1,25(OH)2D and intestinal calcium transport routes. According to the data, 1,25(OH)2D causes both transcriptional and quicker increases in calcium transport. Vesicular transport pathways may play a role in these faster increases. Given that relative dietary shortages are common and that excessive calcium intake is also possible, redundancy in this system likely allows for improved regulation and efficiency of calcium absorption (Wang *et al.*, 2017; Bello *et al.*, 2020; Cao *et al.*, 2020; Hsieh *et al.*, 2021; Nassar & Alotaibi, 2020).

Renal Calcium Reabsorption

The main functional organising unit of the kidney is the nephron. Blood is filtered through the glomerulus, the first portion of the nephron. The distal convoluted tubule, the connecting tubule, the loop of Henle, and the proximal convoluted tubule are all places where the filtered fluid and its contents go after that. Calcium may be reabsorbed in a number of locations along the nephron. The glomerular filtrate contains ionised calcium that makes up around 45% of the total calcium in the plasma. The proximal convoluted tubule undergoes 65 percent or more passive and paracellular calcium resorption. In contrast to calcium reabsorption, which occurs in the proximal tubule due to solvent drag, calcium transport is mostly brought on by calcium after water is reabsorbed as opposed to ion channel action (Cao et al., 2020). In the Henle loop's thick ascending limb, around 20–25 percent of the filtered calcium is reabsorbed. The sodium-(potassium)-chloride cotransporter 2 (NKCC2), the ATP-sensitive renal outer medullary potassium channel (ROMK), and the basolateral sodium/ potassium-transporting ATPase (NaK-ATPase) all collaborate to keep the lumen on the apical (luminal) surface at a positive electrochemical potential (Mensah et al., 2021; Mulualem et al., 2021). Sodium, calcium, and magnesium cations are encouraged to be reabsorbed paracellularly by this potential. The renal tubular epithelial cells in the thick ascending limb of the kidney exhibit basolateral (interstitial) extracellular calcium accumulation that is detected by the G protein-coupled extracellular calcium-sensing receptor (CaSR), which is encoded by the CaSR gene. High levels of calcium cause decreased ROMK expression.

Factors Supporting Calcium Absorption

Vitamin D

Elderly people are much more likely to suffer from vitamin D insufficiency due to a lack of dietary sources of the vitamin,

reduced sun exposure, decreased skin vitamin D synthesis, and decreased kidney vitamin D conversion into its active form. Vitamin D comes in two primary varieties: Ergosterol, a naturally occurring precursor to vitamin D found in plants, fungi, and invertebrates, is converted into vitamin D2 (ergocalciferol), which is created when it is subjected to UV-B light. The second is cholecalciferol or vitamin D3. This is made when the skin is exposed to sunlight, and it comes from a precursor to cholesterol called 7-dehydrocholesterol.

Prebiotics, Probiotics, and Synbiotics

Prebiotics are nondigestible fibres that act as a substrate for gut microbes to metabolise nutrients and energy sources. The survival of gut microorganisms may also be improved by prebiotics. In multiple studies, prebiotics have been proven to improve calcium absorption, which may reduce the risk of osteoporosis. Prebiotics containing fructans, such as inulin, oligofructose, and fructooligosaccharide (at a dose of 4 to 8 g/ day), enhance calcium absorption. The pH in the colon is lowered as a consequence of these fructans being fermented by gut microbes. This may increase the absorption of trace minerals, such as calcium since calcium is transformed into an ionic form in an acidic environment, which makes it more soluble and available. Because of the swelling of the mucosal cells and the acidic environment they generate, the prebiotic fibre fermentation process produces short-chain fatty acids, which improve calcium absorption (Wang et al., 2017; Bello et al., 2020; Ma et al., 2020; Zhao et al., 2020; Baláž et al., 2021; Chen et al., 2021; Gautron et al., 2021; Hsieh et al., 2021). This process also increases the surface area of the gut.

Fat

Fat affects the bioavailability of vitamin D, which is fat-soluble and necessary for calcium absorption. The kind of fatty acids that fat creates directly affects how much calcium is absorbed (Younas *et al.*, 2021). The greatest effective absorption occurred when saturated fatty acids were introduced to the meal. The saturated fatty acids aid in calcium absorption, although their effect on bone health is less significant than that of a diet high in monounsaturated fatty acids or with a typical amount of fat. According to the research, the amount of hepatic cytochrome P450 protein, intestinal calbindine D9k gene expression, and trabecular volume thickness were all significantly larger in mice that were fed a diet enriched in monounsaturated fatty acids than in animals that received an unchanged diet. Thus, a diet high in monounsaturated fatty acids is recommended (Ma *et al.*, 2020).

Importance of Calcium in Daily Life

Bone health

The defining feature of osteoporosis, a complicated disease with several causes, is an asymptomatic decrease of bone mass per unit volume (Nassar & Alotaibi, 2020). Fractures may happen from even small damage when bone mass is too low

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(Cao et al., 2020; Mensah et al., 2021; Mulualem et al., 2021). This is because structural integrity and mechanical support are weakened. Fractures of the ribs, hummers, pelvis, distal radius, proximal femur, and distal radius are more common. Fractures are more common in postmenopausal white women and the elderly (Kobus-Cisowska et al., 2020). The structure of the bone is constantly being repaired or reconditioned and is metabolically active. This process is managed by the cells that produce and resorb bone. Bone growth equals bone resorption in a normal, healthy adult bone, leaving no overall change (Chen et al., 2021). Variations in bone mass are significantly influenced by magnesium, phosphorus, and calcium, the primary mineral ions of bones. These mineral ions need to be present in extracellular fluids in regular and adequate quantities for proper bone calcification and development to occur (Ma et al., 2020).

Hypertension

Both osteoarthritis and hypertension are common in older people, and it is thought that both conditions are caused by a lack of calcium (Wang et al., 2017). Particularly in postmenopausal women, consuming more calcium is thought to reduce the risk of cardiovascular disease. The therapy of hypertension is known to benefit greatly from calcium (Bello et al., 2020). Blood pressure is regulated by calcium as well, and some research suggests that individuals or lab animals with proper calcium levels may be protected against hypertension. When calcium consumption was increased (1-2 g/day), pre-eclampsia and pregnancy-related hypertension were considerably reduced. Calcium supplementation in the diet has been shown to lower systolic and diastolic blood pressure. However, people with low calcium consumption (less than 0.8 g/day) had higher blood pressure. The relationship between calcium and systolic and diastolic blood pressure requires more study (Hsieh et al., 2021). A meta-analysis found that calcium supplementation during pregnancy among women at risk of calcium insufficiency reduces pre-eclampsia incidence by more than 50%. ES calcium has a high bioavailability, so we may expect positive outcomes even if there isn't enough evidence to back up its usage in the treatment of calcium.

Weight Loss

A high-calcium meal consumed during periods of high calorie intake decreased adipocyte lipid deposition and weight gain, suggesting that calcium may play a role in managing body weight. Ca2+ ions inside cells are decreased by calciumrich diets through parathyroid hormone (Cao *et al.*, 2020). The expression of fatty acid synthase would decrease as a consequence of the elevated intracellular calcium. The control of lipid synthesis depends on this enzyme. Lipids are associated with greater weight loss, and the faster oxidation caused by calcium promotes the breakdown of fat tissue. Furthermore, dietary calcium promotes faecal fat excretion, resulting in an increase in faecal fat excretion. You may also take hydroxyapatite as a supplement, which is a mineral found in fish bones and marine shells (Kobus-Cisowska *et al.*, 2020; Mensah *et al.*, 2021; Mulualem *et al.*, 2021). People who eat a lot of calcium have higher plasma levels of the peptide tyrosine, which makes people feel fuller and makes them eat less, which helps them lose weight (Kobus-Cisowska *et al.*, 2020).

Colorectal Cancers

Colorectal cancer is the leading cause of mortality in the West. Dietary calcium is thought to be beneficial for preventing adenomas and colorectal cancer. The binding of calcium to ionised fatty acids and carcinogenic bile acids has been shown to reduce the incidence of colorectal cancer. This hindered the ability of these cancer-causing agents to grow in the colon's mucosal layer. It is also known that calcium may boost the variety and selectivity of colon cells by binding to calcium receptors. This has a variety of biological impacts, one of which is the activation of isozyme protein kinase C. It has been discovered that both in people and animals, colon cancer cells produce this protein differently. Numerous clinical studies have shown that calcium supplementation may support colon cells' defence against pre-malignant alterations. There might be a link between taking calcium supplements and getting cancer at the end of the colon (Zhao et al., 2020; Baláž et al., 2021; Li et al., 2021; Younas et al., 2021; Zhang et al., 2022).

Calcium Signaling

Cells must communicate in order to respond to changing conditions, and this communication necessitates messengers whose concentrations fluctuate throughout time. Calcium ions have a profound impact on a cell's life and death. Many proteins interact with calcium ions, which changes how they behave, associate, and localise. Calcium serves as a universal dispatch rider for almost all cell types, including immune system-related T- and B-lymphocytes and mast cells. Also, the body uses calcium signalling for a number of things, such as growth and proliferation, differentiation, and programmed cell death (apoptosis) (mRNA), which is the process of making copies of DNA into new molecules (Nassar & Alotaibi, 2020).

General Methods of Calcium Extraction

ESs are among the most abundant biomaterials found in nature. They are thrown away after being used with egg yolk and albumin, hence constituting a significant amount of waste created by the food industry (Mulualem *et al.*, 2021).

Pulsed Electric Field (PEF)

Recently, the food and pharmaceutical sectors have paid a lot of attention to the revolutionary extraction technique known as the pulsed electric field because of how affordable it is (Abo El-Maaty *et al.*, 2021). This technique was first used as a non-thermal way to inactivate bacteria and enzymes at room temperature in order to increase the value of food and medicinal materials (i.e., it does not raise the temperature of the food during processing) (Wang *et al.*, 2017; Bello *et al.*, 2020). In addition to inactivating food item enzymes, PEF may be used to extract and recover high-value chemicals from food molecules and to improve the stability and safety of food. Through this process, the structural and functional properties of the molecules are also somewhat altered. PEF's electroporation method provides the basis for the extraction of valuable chemicals (pore formation in the membrane). It causes the release of the cell sap, which is used to empty cells. The main benefit of PEF is that it is uniform, which is possible because all of the cells, inside and out, are treated (Nassar & Alotaibi, 2020).

High Intensity Pulsed Electric Field (HIPEF)

HIPEF, which employs high-voltage pulses delivered between two electrodes, is an extraction technique similar to PEF. The HIPEF technique may be used in the food industry since it produces a large volume in a short period of time (Cao et al., 2020). The ES calcium malate was extracted using this technique. Incorporating malic acid into 1 g of ES powder (50 mL). The mixture was added to the HIPEF extraction apparatus at a rate of 25 mL per minute, and the pulse generator was activated for 120 seconds. The product was removed and then centrifuged to separate the solid from the liquid. The production of calcium malate, which improves calcium absorption in vitro, has been shown to be energy-efficient, simple to use, and economical using HIPEF technology (Baláž et al., 2021). Even though PEF is cost-effective, it is hard to run, doesn't work all the time, and can only treat a limited number of people (Mensah et al., 2021).

Electric Discharge-Assisted Mechanical Milling (EDAMM)

ES powder is supplied between two stainless steel electrodes in a reaction tank for EDAMM equipment. While another shakes the ES to break it apart at a frequency of 10 Hz, one electrode maintains its position. The ES was altered by using an alternating current and vibrations to produce an electric discharge between stainless steel electrodes and the chamber walls. In this experiment, ES is grounded in atmospheres including air, argon, and nitrogen for a total of 15 minutes. Most of the calcite to calcium oxide conversion will take place in the presence of argon. The EDAMM method allows for complete calcium oxide recovery in under 15 minutes (Bello et al., 2020). Mechanical milling has been used to effectively produce a number of chemical reactions and fine ceramic and metallic powders. Reactive compounds, catalysts with a high surface area, quasi-crystalline, amorphous, and non-crystalline materials, as well as certain supersaturated solid solutions, are among the byproducts of milling processes (Wang et al., 2017). Using low current and high voltage, EDAMM generates quicker reactions as well as cutting-edge synthesis and processing capabilities. Only 15 minutes after using EDAMM can the calcium oxide be completely restored. Using standard calcium carbonate calcination, comparable results were produced. A high temperature of 900 °C is required to transform calcium carbonate into calcium oxide (Ma et al., 2020).

High Energy Milling (HEM)

In the domains of powder metallurgy and mineral processing, milling is a common technique when employing metal powders to manufacture a range of products (Abo El-Maaty *et al.*, 2021). HEM involves converting big molecules into smaller ones while retaining their clumping characteristics (Hsieh *et al.*, 2021). HEM is a well-known technique for altering the structure and characteristics of materials. Thermal analysis may be used to pinpoint these alterations, which are caused by the accumulation of mechanical energy (Nassar & Alotaibi, 2020). ES powder is processed by mechanical activation and high-energy milling. The mill is filled with 50 10 mm tungsten carbide balls and 5 g of ES (Ma *et al.*, 2020). When there is air present, it rotates at a rate of 500 revolutions per minute for around 480 minutes. The machine was halted after every milling cycle (which lasted somewhere between 1

Table 1: Patented research for egg shells

Title of Patent	Application number	References
ES calcium liquid	JPH 06303949A	Kuwataka, 1994
ES calcium composition	JPH 0956363A	Suguro <i>et al.</i> , 1997a
Manufacturing method for oxygen ES calcium powder	TW200820908A	Liu, 2008
Method of preparing calcium propionate using ES	CN1288125C	Xiaoyan & Deyu, 2006
ES calcium composition and its production and food containing the ES calcium composition	JP2000093123A	Suguro <i>et al.</i> , 2000
Container structure of active oxygen ES calcium	TWM314638U	Liou, 2007
ES calcium composition	JPH 09220071A	Suguro <i>et al.</i> , 1997b
ES calcium oxide powder and preparation and application there of	CN101209112B	Fayong, 2011
A kind of method preparing composite organic acid calcium magnesium salts with ES, shell and	CN104223100B	Jiwu <i>et al.</i> , 2016
magnesium oxide		
Calcium preparation obtained from the ES	PL238906B1	Frąckowiak <i>et al</i> ., 2021
Method for manufacturing ESs calcium solubility in water using ESs	KR20150028061A	Hyun, 2015
A method of liquid calcium acetate fertilizer is prepared using ES	CN109912319A	Baozhong & Wang, 2019
Calcium absorption enhancer	TW200916107A	Matano <i>et al.</i> , 2009
Method for preparing novel organic calcium using ES	CN101024605A	Haizheng & Junde, 2007
Highly active calcium oxide and powdering agent	JPH 0717711A	Shirane, 1995
Egg white peptide chelated calcium and preparation method thereof	CN113575966A	Qun <i>et al.,</i> 2021
Egg-shell ion powder and method for producing the same	JP2006246706A	Sato, 2006
ES calcium malate chewable tablets and preparation method thereof	CN102150776B	Songyi <i>et al.</i> , 2014
Method for fabricating calcium phosphate and calcium phosphate fabricated by using the same	W02007126211A1	Park, 2007

and 480 minutes), and 0.5 g of ES powder was taken out and marked with the time.

Patented Products of ES Calcium

Using shell and ES membranes has started the process of creating new biomaterials. The production of bioactive materials based on calcium phosphate (CaP) structures using ES as the main calcium source has been used for several therapeutic applications (Ma *et al.*, 2020; Chen *et al.*, 2021; Gautron *et al.*, 2021). Table 1 provides a summary of the patented research.

CONCLUSION

The ES membrane is a promising natural biomaterial with applications in the sectors of nutraceuticals, medicine, bioremediation, assisting chemical processes, and cosmetics. Despite their numerous advantages, ES membranes cannot be changed in terms of their size, shape, or thickness, and the fact that they are insoluble because of disulfide limitations in their molecular structure severely restricts the range of uses for ES membranes. However, soluble ES membrane protein (SEP) has been recommended as an alternative biomaterial in various studies. SEP is created by dissolving the ES membrane and altering its form, thickness, and solubility by the reductive breakage of disulfide bonds. SEP has been extensively used in tissue engineering in the biomedical sector. Since Ovocalyxin-36 (OCX-36), a new possible antibacterial protein connected to the ES membrane, was just found, there is also a chance that ES membranes will be used in the pharmaceutical business in the future.

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