

1

3,194,732

ASSISTING HEALING OF SKIN-DENUDED AREAS ON THE BODY WITH DRIED NON-FIBROUS EGG-SHELL MEMBRANE PRODUCTS AND COMPOSITIONS THEREFOR

Irene Neuhauser, 5759 S. Kenwood, Chicago 37, Ill.

No Drawing. Filed Oct. 3, 1960, Ser. No. 59,804

5 Claims. (Cl. 167—58)

This application is a continuation-in-part of my application Serial No. 845,104, filed October 8, 1959, now abandoned.

This invention is directed to new and useful methods for stimulating and assisting healing processes in damaged mammalian tissues as, for example, in areas of the human body where tissue has been lost or damaged as a result of injuries such as abrasions, cuts, burns, ulcerations and from surgical procedures and it is also concerned with the preparation of new and useful materials for effectively achieving these objectives.

Numerous approaches have heretofore been made and much study has been directed to problems involved in bringing about healing of areas where skin has been wholly or partially lost and where underlying tissues may have been damaged or removed as a result of burns, cuts, abrasions, ulcerations or from surgical intervention. The nature and character of the treatments currently used vary with techniques and preferences of individual surgeons and are dependent, also, on the nature, surface conditions and extent and depth of tissue involvement.

A commonly employed procedure is that of viable skin grafting, in which the surgeon transplants shavings of living epidermal tissue from undamaged areas after suitable preparation. Such grafts have varied in size and thickness of the skin shavings used for grafting and in other details of procedure. Thiersch grafts, Ollier-Thiersch grafts, Wolfe grafts, pinch grafts, full-thickness and split-thickness grafts, and stamp grafts, are names associated with some of the various types of skin grafts and procedures which have been used. In preparing skin shavings for grafts, the surgeon attempts to include portions of the stratum germinativum of the epidermis by cutting across the "rete pegs." When such grafts become attached to the surface tissues of the damaged area, they may become vascularized and form islands or centers of epithelialization on the surface of the lesion. Grafts for which the patient is the donor of the grafted skin are known as autografts.

It frequently happens that the size of the lesion is such that not enough undamaged skin areas remain on the patient to provide autografts to cover the entire surface of the lesion. In such cases a portion of the lesion may receive an autograft and the remaining damaged area may receive grafts of living skin from another person, or of dead skin from a cadaver. Although such grafts, known as homografts, do not persist and do not become finally a part of the skin covering the healed lesion as autografts frequently do, they accomplish many desirable results such as preventing fluid loss, repressing the excessive development of granulation tissue, decreasing pain and stimulating growth of the stratum germinativum of the epidermis at borders of the lesion. Eventually homografts are rejected, that is, they slough off. It may be possible by then to cut additional skin grafts from the now-healed donor sites and so eventually to cover the lesion completely with viable autografts. Considerable effort has been directed to bring about the permanent survival of viable homografts but only slight progress has been achieved and no clinical applications of any of these studies have been widely accepted.

Although the homograft does not persist and become

2

a part of the skin over the healed lesion it is of great value as a practical measure since, by use of homografts together with autografts, lesions with greater areas and degrees of tissue damage may be satisfactorily treated.

However, the limited availability of human skin in relation to the needs for homografts makes it important that other more readily available materials, capable of performing one or more of the functions of the homograft, be found and that practical procedures for their preparation and use should be developed.

An area of approach which has been extensively studied, extending back approximately eighty years, has involved the utilization of egg-shell membranes, the membrana testacea of eggs of domestic fowl. Numerous workers have utilized egg-shell membrane as a substitute for skin grafts but with varying degrees of success. In certain cases, where fresh egg-shell membrane was used in the repair of ruptured ear drums and as a graft to damaged areas from which skin had been entirely or partially lost, it was stated to have achieved reasonable success. In some cases, reported by other workers, fresh egg-shell membrane was found to be ineffective. Certain physiologists and surgeons concluded that satisfactory results could be obtained provided (1) that the egg-shell membrane pieces were applied with that surface towards the surface of the lesion which was previously in contact with the egg shell, and not the reverse, and (2) that the area was scarified until bleeding was produced prior to the application thereto of the egg-shell membrane. Unless these rules were carefully observed, according to certain of the workers in the art, the egg-shell membrane would not become "attached organically" to the denuded area to which it was applied. Another important criterion for the proper utilization of egg-shell membrane, according to early workers in the art, was to insure that, when applied with the shell-side to the lesion, it be in contact with the proliferating cells of the stratum germinativum of the skin at the periphery of the lesion. The said workers reported that the egg-shell membrane itself, and of its own initiative, takes no part in the formation of connective tissue but that the egg-shell membrane substance is transformed into connective tissue gradually, there being an interpenetration of the proliferating tissue into the structure of the membrane, followed by the establishment of circulation to care for the newly formed connective tissue structures.

Other workers have insisted that egg-shell membrane be applied with the albumen side toward the lesion, an exact reversal of the opinion of the above mentioned workers. The basis for this was the belief that the inner side of the inner shell membrane contained nucleated cells which served as foci for the healing processes. It is now known that such nucleated cells do not exist in egg-shell membranes. Still other workers employed the shell membranes from boiled eggs but disregarded the question of which side was placed in contact with the lesion.

Despite repeated investigation of the possible use of egg-shell membrane as a substitute for skin in grafting, the practice has not come into any appreciable or significant use. The difficulties of separating egg-shell membrane from eggs, the difficult problems of handling moist membrane pieces and the indifferent success which has attended the use of egg-shell membrane as well as other factors, have all combined to make such approaches of dubious practical value.

In accordance with the present invention, highly important and significant improvements have been made which render the use of egg-shell membrane of great practical importance. It has been found, among other things, in accordance with the present invention, that egg-shell membrane can be treated in such a manner

to convert it into such forms as to make its use highly practicable and of great value in meeting problems encountered in connection with successful clinical management of skin-denuded areas, deep-tissue wounds and lesions which are refractory to the usual treatment procedures. These have heretofore frequently required the utilization of autografts and to a lesser extent, and with less success, the use of homografts. The present invention makes possible the production of novel egg-shell membrane products, which are highly effective for use in place of human skin in skin grafting operations, which are easily manipulated in use and which can be made by simple practical procedures. Moreover, in the various forms in which the novel egg-shell membrane products are produced, in accordance with the present invention, depending on the type and condition of the lesion to which they may be applied, they frequently display additional desirable properties such as a strong hemostatic action which tends to arrest bleeding, and a soothing, pain-relieving action, a type of action which some workers have reported to be a conspicuous property of homografts of human skin. Moreover, in the various forms in which the novel egg-shell membrane products are produced, in accordance with the present invention, they are readily and effectively storable so that they can be stockpiled in any desired amounts to meet emergency clinical situations which might be created due to holocausts, such as extended fires, wars and the like. These products constitute an effective treatment procedure providing a number of desirable characteristics and properties only some of which have heretofore been obtained by grafts of human skin.

The novel egg-shell membrane products made in accordance with the present invention play exceedingly important roles in tissue healing and grafting procedures. They serve, in fact, as effective biological dressings. In addition, by reason of their stimulating the regeneration of epithelial tissues and by providing topically certain factors which are essential aids in the natural processes of tissue repair, they appear actively to promote the regeneration of tissues by stimulation and possibly by providing essential components for synthesis of the protein of the regenerated tissues.

The present invention, in broad terms, involves the production and utilization or treatment of separated particles, mainly non-fibrous in form, prepared or derived from egg-shell membrane. Various effective procedures by which such may be accomplished are described in detail below.

Pursuant to one aspect of the present invention, egg-shell membrane is washed and dried and then ball milled to convert it into a pulverulent or powder product which is essentially non-fibrous in form. These essentially non-fibrous products may consist solely of egg-shell membrane material or they may consist of mixtures of the egg-shell membrane material with various other materials. The egg-shell membrane essentially non-fibrous product may, for example, be admixed with fibrous materials such as cotton or cellulosic fibers and deposited as a coating or coatings on certain desirable base materials as, for instance, cellulose or alpha-cellulose or regenerated cellulose or cellulose acetate or nylon or similar natural or synthetic textile fabric or plastic base materials, woven or non-woven, in sheet form, perforated or imperforate, and, if desired, the egg-shell membrane non-fibrous product may be applied to either one or both sides of the base materials. Furthermore, if desired, the egg-shell membrane essentially non-fibrous materials may be bonded to the base material through suitable adhesive compositions such as compositions containing pectin, gelatin, starch and starch derivatives, innocuous vegetable gums, or like innocuous adhesive materials. Typical of such felts or sheets are those made from mixtures, in varying proportions, of uncooked or cooked egg-shell membrane essentially non-fibrous ma-

terial with cellulosic fibers, for instance, 35% to 65% alpha cellulose fibers, balance said egg-shell membrane essentially non-fibrous material.

In another, and particularly preferred, aspect of my invention, instead of converting the egg-shell membrane essentially non-fibrous material into dry felt or sheet form, as indicated above, said egg-shell membrane material may be directly used, in the practice of my invention, in its particulate solid form which provides large surface area per unit of weight.

In those instances where the egg-shell membrane essentially non-fibrous materials are mixed with other materials, for example, cellulosic materials such as alpha cellulose in fiber form, such latter materials should advantageously amount to not more than 75%, and, better still, not greater than 50%, of the dry weight of the total of said material and said egg-shell membrane essentially non-fibrous material. The cellulosic or like fibers or other materials may be admixed with the egg-shell membrane essentially non-fibrous material in aqueous suspension and the resulting fiber pulp mixture may then be laid down to form the felted sheet, or a coating or layer on a woven or non-woven base. Alternatively, a preliminary felted sheet may be laid down from a cellulosic or other fiber pulp and the essentially non-fibrous egg-shell membrane material deposited thereon or embedded in, or intimately mingled therein. Analogously, mixtures can be made where the egg-shell membrane essentially non-fibrous materials are admixed with other particulate solids to produce final products in the form of pulverulent or powdered products having low bulk densities.

The new and useful egg-shell membrane products contemplated by the present invention encompass dry particulate materials having a low bulk density, generally in the range of 0.05 to 0.40 grams per cubic centimeter, depending upon the particular manner of preparation, and a very large area of surface for a given weight of material, all as hereafter described in detail. In the form in which they are used, they are either essentially non-fibrous in character or, if they do encompass, include or comprise particles of a fibrous character, the nature or sizes or shapes or proportions of the fibers are such that said membrane products are incapable of being laid down, without the addition of other fiber materials, to form dried felts or sheets which are form-sustaining and which can be handled reasonably without crumbling or disintegrating. The egg-shell membrane products here contemplated may, however, as indicated, produce form-sustaining dried felts or sheets which can be handled reasonably without crumbling or disintegrating only if they are admixed with a sufficient quantity of fiber materials of a character such as in themselves, without the addition of the egg-shell membrane products, are capable of producing such latter felts or sheets utilizing techniques such as are known in the art for the production of felts or sheets from fiber pulps. The term "non-fibrous," as applied to the egg-shell membrane products of the present invention, will, accordingly, be understood to mean products such as have been characterized in this paragraph and wherein the form or shape of the particles, as distinguished from the internal structure of the particles, are non-fibrous.

In the practice of the present invention, egg-shell membrane is desirably washed and dried and then reduced to exceedingly small mainly non-fibrous particles. This is conveniently and very advantageously done in a high speed homogenizing apparatus. I have found that excellent results are obtained with a homogenizer such as a "Vir Tis" or the like, the cutting blades of which can be caused to rotate up to a speed of approximately 45,000 r.p.m. Other types of equipment can, however, be used. The usual "Waring" blenders and the "Osterizers" employed in the home for preparing dispersions and for disintegration and emulsification of food products have been

found to give satisfactory results, especially if the rotating blades are first sharpened. Any other type of equipment may be employed so long as it and the details of procedure used serve to convert the egg-shell membrane into finely divided mainly non-fibrous form. For example, dried egg-shell membranes may be converted into finely divided particles having mainly a non-fibrous form by ball-milling the dried egg-shell membranes; or the dried egg-shell membrane may be finely subdivided into particles having mainly non-fibrous form by subjecting the dried egg-shell membranes to the action of a high speed homogenizer while the membrane pieces are suspended in air or in other suitable gases, or in a liquid which does not soften, or does not appreciably soften, the dried egg-shell membrane as a result of imbibition of the liquid by the dried egg-shell membrane. Examples of such liquids are cyclohexanol, aniline, N-N-dimethyl formamide, water-free methyl-ethyl ketone (2-butanone), 1:4-dioxane, benzthiazol, and pyridine. Numerous other nonaqueous liquids which are not imbibed by dried egg-shell membrane can be used, those above mentioned being simply illustrative.

After homogenization or disintegration of the egg-shell membrane, the finely divided mainly non-fibrous particles can be readily separated from the suspending liquid employed. This step is accomplished by filtration, by centrifugation, by evaporation of the suspending liquid or by other well-known procedures for separating solids from liquids. The adhering residue of suspending liquid, if any, is then removed from the egg-shell membrane particles by washing the particles with suitable solvents and these latter are removed by filtration, centrifugation, evaporation or other convenient means. Care must be taken not to squeeze or compress the disintegrated or homogenized membrane mass as this tends to cause the formation of hard aggregates on drying. The resulting dry solid material consists of aggregates of small particles of disintegrated egg-shell membrane. The dry aggregates of the mainly non-fibrous particles are easily disaggregated into separated mainly non-fibrous particles by acting on the above described aggregates in the homogenizer in the absence of any suspending liquid, the aggregate pieces being disaggregated in air, or in other suitable gases, by high speed action of the homogenizer. The dry, pulverulent mass of separated air-dispersed or gas-dispersed mainly non-fibrous particles is now removed from the homogenizer. The disaggregation of the dry mainly non-fibrous aggregates in the homogenizer or other suitable high speed disintegrator requires but a short period of action at high speeds. Prolonged subjection of the products to treatment in the disaggregating apparatus at high speeds may build up elevated temperatures in the product which could damage it.

Pursuant to one embodiment of the present invention, the mainly non-fibrous disaggregated disintegrated egg-shell membrane substance may be used for the therapeutic purposes previously described.

Pursuant to another embodiment of my invention, the above described mainly non-fibrous disintegrated disaggregated egg-shell membrane, as previously described, may be applied as a coating to cotton gauze, to paper, to woven and to non-woven fabrics, to plastic sheets both perforated and imperforate, or to other desirable base materials, on one or both sides, and the egg-shell membrane products may be made to adhere, if desired, to the surface of the base materials by means of suitable adhesive compositions, such as compositions containing pectin, gelatin, starch and/or starch derivatives, vegetable gums or soluble synthetic resinous polymeric substances and like innocuous adhesive materials.

The egg-shell membranes which constitute the starting material used in carrying out my invention can be separated from egg shells in any convenient manner. Thus, for example, this may be accomplished in a purely mechanical manner as, for instance, by rolling and pulling

the membranes away from the washed egg shells after removal of the yoke and albumen of fresh or uncooked eggs. In the case of cooked egg-shell membranes, these, too, may be removed by pulling them away from washed egg-shells which have been placed, for example, in boiling water to effect such cooking. In either case it is desirable to effect the separation of the egg-shell membranes from the egg-shells by grasping an edge of the membranes and rolling or pulling them away from the calcareous part of the egg shells.

Instead of employing purely mechanical means for effecting separation of the membranes from the egg shells, a combination of chemical and mechanical means can be utilized. One convenient procedure is to agitate, in a churn or the like, water-washed, very coarsely chopped or shredded egg shells containing the adhering membranes with several times their weight of a dilute acid, for instance, a 0.25 to 4% solution in water of mineral acids such as hydrogen chloride, or a 2 to 15% solution in water of organic acids such as formic acid, or acetic acid or lactic acid or propionic acid, for a period of time, generally one to several hours, agitating until the membranes are loosened and fall away from the egg shell particles. The membranes may then be separated from the egg shell pieces by counter-current washing with water or aqueous solutions and by decantation steps. In case a small proportion of shell particles still remains attached to some membrane pieces, the membrane pieces are collected, washed with water by counter-current procedures and drained, then agitated for a period of one to four hours with a small amount of the dilute acid solution, followed by separation of the acid solution and counter-current washing of the membranes with decantation from bits of sedimented shell, using several changes of water.

The separation of the membrane from the shell may be effected with carbonic acid, that is, with an aqueous solution of carbon dioxide under pressure, though the process is time consuming. The time needed in this process may be decreased by agitating the shell pieces and by including with the shell material a cation exchange resin, for example, a cross-linked polystyrene polysulfonic acid, in the free acidic or hydrogen form, while the shell pieces are mixed with a solution of carbon dioxide in water, under compressed carbon dioxide. In these cases the separated membranes are finally washed with water or aqueous solutions using countercurrent washing and decantation steps and the undissolved shell pieces and the cation exchange resin particles are separated with suitable sieves. The resin can then be regenerated to the free acid or hydrogen form by mineral acids, preferably hydrochloric acid, and is then ready for re-use.

In separating the membrane from the calcareous portion of the shell by means of dilute acids, separation is practically complete long before complete solution of the mineral part of the shell has taken place. It has been noted that the amount of mineral matter dissolved during the period necessary for membrane separation is much higher in the case of boiled egg shells than with uncooked egg shells, although the time of acid action is essentially the same. Evidently, boiling has created conditions in the shell which make more rapid the dissolving of the mineral matter by dilute acids.

The separated spent organic acid solutions may be regenerated for re-use conveniently by passing them through a column of appropriate cation exchange resin in acidic or hydrogen form and the cation exchange resins themselves may be regenerated to their acid forms with mineral acid solutions, such as dilute hydrochloric acid. Previous removal of the major portion of the dissolved calcium from the spent acid by the addition of sulfuric acid in amounts just insufficient to combine with all the calcium present and subsequent separation of the precipitated calcium sulfate by decantation and filtration, makes further regeneration of the dilute acid filtrate by treatment with

cation exchange resins in hydrogen form a more efficient and economical procedure.

Another suitable way of effecting separation of the membranes from the egg shells is illustrated by the following procedure. Approximately 37 grams of water-washed egg shells containing adhering membranes are chopped and placed in an acid resistant (for example, enameled) steel pressure vessel together with 1200 ml. of 6% w./v. acetic acid. The air in the vessel is displaced by carbon dioxide gas and then a pressure of 450 to 500 p.s.i. of carbon dioxide gas is maintained in said vessel for one hour. The carbon dioxide is then removed and suction by vacuum means is applied to the vessel. The suction is cut off, applied again, and again cut off. The vessel is then opened and the contents placed in a jar and agitated, any gases evolved being allowed to escape. The membranes which separated from the egg shells are removed by decantation. Repeated washing with water is followed by decantations between washings which result in effecting removal of substantially all of the membranes from the egg shells and in removing all of the shell particles from the membranes.

The egg-shell membranes, after removal from the egg shells, are desirably washed in water or in aqueous media. For convenience in storage or subsequent handling, it is desirable to subject them to a partial or complete drying operation depending on the physical form of the product desired from the subsequent homogenization or disintegration step. If the membranes are to be disintegrated, homogenized or pulped, for example, in a non-aqueous medium for the production of mainly non-fibrous egg-shell membrane particles, a partial removal of the water contained in the washed membranes may be accomplished simply by pressing the drained membranes between absorbent surfaces, for instance bibulous paper such as blotting paper. In this form the "blotter dry" egg-shell membranes contain about 30% by weight of membrane material, the balance being water, and they may be stored under refrigerated or freezing conditions for subsequent use in accordance with the teachings of my invention. Alternatively, the drained membranes may be dried in air or in a vacuum drier and such dried membranes may be stored for prolonged periods, indeed almost indefinitely, at room temperatures without deterioration. They may be used in this form in the subsequent disintegration or homogenization step.

The following examples are illustrative of procedures which are useful in the production of egg-shell membrane mainly non-fibrous products pursuant to my invention. It will be understood that various changes can be made in the light of the teachings disclosed herein without departing from the essential guiding principles, and particularly with respect to translating the procedures to commercial scale operations.

Example 1

10 grams of "blotter-dry" mechanically separated egg-shell membranes (equal to about 3 grams dried membrane) which prior to "blotter-drying" are previously washed with water, are cut up into thin strips about $\frac{3}{16}$ to $\frac{1}{4}$ inch wide and $\frac{1}{4}$ to $\frac{3}{4}$ inch long and air-dried, then placed into a laboratory ball mill containing alundum balls and ball milled until an essentially non-fibrous powder product results. Its bulk density is 0.31 gram per cubic centimeter.

Example 2

An alpha cellulose fiber aqueous suspension prepared by homogenizing 1 gram of filter paper in 175 ml. water is poured onto a stainless steel screen box having 200 openings to the lineal inch and the water is allowed to drain therethrough. The resulting felted mass of cellulose fibers is then sucked down with suction and 1.2 grams of the egg-shell membrane product produced pursuant to Example 1, suspended in 175 ml. water, is poured over the surface of the felted mass of cellulose fibers. The suction

is stopped and then 10 ml. of a 10% w./v. water solution of glycerol is carefully poured over the mass, which is in the form of a sheet, suction is then re-applied, and a top plate of aluminum is pressed down on the sheet to force out as much liquid as possible. While keeping the top plate in place, the screen box is placed in inverted position on top of a conical flask so that the weight of all is supported by the pressure of the rim of the top of the conical flask against the plate. The resulting set-up is allowed to stand overnight to allow evaporation of water through the screen interstices and the plate is then removed leaving a dry sheet of felted alpha cellulose fibers containing said egg-shell membrane product on one side. Alternately, the suspension of alpha cellulose fibers and egg-shell membranes product produced pursuant to Example 1 may be mixed together before pouring the mixture on the screen box. This results in a sheet with both sides essentially similar and with the egg-shell membrane product distributed through the sheet.

The glycerol, which is desirably utilized, serves as a humectant so as to inhibit undue embrittlement or drying out of the felts or sheets of the felted fibers containing said egg-shell membrane product. In place of glycerol, other humectants, generally aliphatic polyols, can be used, typical of which are sorbitol and propylene glycol. The humectant can be incorporated at any suitable stage of the process.

Example 3

This example is carried out in the manner described in Example 1 except that the starting egg-shell membranes are separated from the egg shells by the acetic acid pressure procedure described above.

Example 4

1.5 grams of air-dried very coarsely chopped pieces and strips of uncooked egg-shell membrane are put in the 250 ml. Vir Tis flask and then the machine is operated at 45,000 r.p.m. without any ice or water cooling of the flask, care being taken to prevent, by careful adjustment, that no material collects in the seal at the top of the flask, there to cause friction between the stationary cap (Teflon) and the rotating shaft cup (stainless steel) and so become scorched. As the operation proceeds, the pieces become disintegrated progressively into smaller bits but after 15 minutes no further progress to smaller particles seems to occur. The machine is stopped and the contents of the flask are examined. The product is a light fluffy particulate material which has an apparent bulk density of 0.555 gram per cubic centimeter. The product consists mainly of pieces of egg-shell membrane and particles of egg-shell membrane but also contains numerous individual free fibers as well as a few matted agglomerates of these freed fibers. The particles are much larger than the particles obtainable by ball milling the same kind of starting material. The freed and agglomerated fibers constitute perhaps 5% to 10% of the total product.

Example 5

1.25 grams of air-dried, very coarsely shredded or sliced pieces of uncooked egg-shell membrane are placed in the 250 ml. flask of the Vir Tis and 170 ml. of anhydrous methyl-ethyl ketone (2-butanone) are added. There is no apparent softening of the crisp membrane pieces indicating little if any imbibition of the liquid into the structural elements of the membrane. The Vir Tis machine is operated at gradually increasing speeds and finally for 12 minutes at 45,000 r.p.m. while the flask is cooled with an ice-water bath. The pieces of membrane rapidly disintegrate and in 12 minutes the agitated liquid looks like milk. It settles rapidly, however, giving a sediment of colorless powder and a hazy supernatant of methyl-ethyl ketone. The sediment product appears to be composed of about equal parts of pieces of membrane and pieces of fibers. After filtration, washing out of

methyl-ethyl ketone with dry acetone, or merely allowing the residual methyl-ethyl ketone to evaporate spontaneously from the collected disintegrated membrane material, an excellent particulate membrane material is obtained of great surface to weight ratio for the topical treatment of wounds. Bulk density of the disaggregated, air-dispersed material is 0.14 gram per cubic centimeter.

The product may also be filtered off from the methyl-ethyl ketone and added to a water suspension of 1.0 gram of alpha-cellulose fiber pulp. The mixture may then be shaken to obtain even distribution and poured into the previously levelled screen box used for making sheets. A good serviceable sheet consisting of a mixture of alpha-cellulose and egg-shell membrane is readily formed in this manner, having an area of 16 square inches.

Instead of removing the suspending liquid from the egg-shell membrane mainly non-fibrous pulp suspension, the suspending liquid may be conveniently removed by subjecting the suspension of egg-shell membrane mainly non-fibrous material to commercial drying, particularly spray-drying procedures, such as are used for the recovery of heat-labile solids from aqueous and other suspensions or solutions. The dry egg-shell membrane fiber aggregates resulting from the spray-drying operation may then be converted to an extremely light pulverulent product by subjecting these aggregates to the previously described disaggregative procedure for the preparation of the extremely light pulverulent product.

I have given the above several examples which will serve to illustrate various of the many types of procedure. It is to be understood that the invention itself is not limited thereby as additional procedures will be apparent to those skilled in the art.

The following table shows the results of several experiments on egg-shell membrane disintegration utilizing various organic suspending liquids in the homogenizer. The coarsely chopped membranes in the suspending liquid were subjected to the action of the homogenizer operating at a speed of 45,000 r.p.m. Homogenization was carried out in the small receptacle using 80 ml. of liquid and the indicated quantity of air-dried membrane. The temperature was controlled by a water bath or by an ice bath around the receptacle and the contents of the receptacle were thus kept from temperatures above 50° C.

Suspending Liquid	Weight of Air Dried Membrane Pieces, g.	Time of Disintegration, min.	Description of Product Formed
1. Anhydrous Pyridine; not imbibed.	0.75	3.5	Product consisted mainly of small particles; a few fiber pieces were seen, frequently stuck together. Some bits or pieces of membrane seen of size in microns: Large 25 x 19, Small 16 x 13. Fiber pieces 6 x 16 when moist.
2. 2:4-Pentandione; not imbibed.	0.75	3.5	Product essentially a powder. A few broken bits of fibers seen. Powder particles size in microns: Large 47 x 53, Small 19 x 38. Fiber pieces 6 x 16 when moist.
3. Chloroform, U.S.P.; not imbibed.	0.75	6	Product essentially a powder. Small particles were 12 x 12 microns or less and numerous. Average particle 14 x 14 microns. Largest bit 120 x 84 microns. A few fibers were seen; longest 132 microns, others 12 to 36 microns long.

The egg-shell membrane products obtained from experiments 1, 2 and 3 in the foregoing table can be effectively utilized in the preparation of the powder form of egg-shell membrane products in the manner described above. The egg-shell membrane products shown in experiments 1, 2 and 3 in the foregoing table can be filtered off, washed with acetone to remove suspending liquid,

air-dried and disaggregated and air-dispersed in the homogenizer to give the powder form of egg-shell membrane product according to the procedure described herein.

The manner in which the dried egg-shell membrane particulate mainly non-fibrous materials of my invention are used to assist the healing of denuded areas on the human body is dependent, in part, on the nature of such areas. Thus, for example, if in the areas the depth to which the tissue has been lost is relatively superficial, a felted sheet containing the egg-shell membrane material in essentially non-fibrous form, or particulate egg-shell membrane product in essentially non-fibrous form described above, is simply placed over said area to cover same. To this end the sheet may be cut to conform in contour to that of the area to be covered. A protective cover of hospital gauze or the like may be laid gently and preferably relatively loosely over the sheet to protect the treated lesion and to prevent contamination.

If the denuded area is quite deep, as in the case of the surgical removal of an ulcer such as a necrotic ulcer, then the area is desirably packed with the dried egg-shell membrane mainly non-fibrous material. To this end, the 100% egg-shell membrane mainly non-fibrous material can be packed or lightly tamped into the area throughout its depth. In this type of situation it is particularly desirable to pack the area with the dried egg-shell membrane material as produced in disaggregated particulate powder or non-fibrous form. The area is again protected from contamination by hospital gauze as described above. As healing proceeds, additional amounts of the dried egg-shell membrane mainly non-fibrous materials may be applied to the denuded area as may be indicated by the rate and degree of healing.

The felted fiber fabrics or sheets containing the egg-shell membrane material in essentially non-fibrous form, and the egg-shell membrane powder or non-fibrous particulate products produced in accordance with my present invention function, when utilized in the manner described above, as protective barriers for the denuded areas, exercising a mechanical protective action as well as markedly assisting in the healing of the lesion. The body fluids present in the tissues of the lesion may become intimately admixed with the particles of the egg-shell membrane mainly non-fibrous products and on drying form a reinforced crust having considerable mechanical protective strength. Further, the enzymic substances present in such body fluids and tissue exudates may act on the said egg-shell membrane particles, presenting as they do an extensive surface area for enzyme adsorption, and such enzymic action may result in the formation of substances which facilitate the healing process and/or stimulate the growth of repair tissues and ultimately of new skin covering. The dried egg-shell membrane mainly non-fibrous products produced pursuant to my invention are characterized by exceptionally great surface area per unit of weight. This fact appears to play some role in relation to the manner in which said materials of my invention function in assisting the healing processes here involved, with due regard for the fact that the exact mechanism of the actions concerned in such processes is presently unknown.

In a typical case, an epithelioma was treated with X-rays and the resulting lesion, on the forehead of a woman, was treated by the application thereto of particulate dried uncooked egg-shell membrane product such as produced pursuant to Example 1 hereof. The lesion had a diameter of about 1 inch. Said lesion, under said treatment, was found to be completely crusted over at the end of one week, and healing of the area was complete in two weeks. Standard techniques usually require healing periods of six to seven weeks as this type of lesion is known to be very slow to heal.

It should be understood that, while it is preferred to utilize uncooked egg-shell membranes in the production

of the novel egg-shell membrane products of my invention, cooked or partially cooked egg-shell membranes can be treated in the same manner as described above in connection with the uncooked egg-shell membranes to produce the egg-shell membrane mainly non-fibrous products as well as to produce felts or felted fiber sheets or other essentially two-dimensional forms containing said mainly non-fibrous egg-shell membrane products, such as have been described herein above.

It has also been found that aqueous suspensions of the egg-shell membrane mainly non-fibrous products, prepared as described above may be treated in aqueous media with enzymes, preferably purified, of proteolytic character, such as papain, ficin, pepsin, trypsin and trypsin related enzymes, bromelin and other proteolytic enzymes of vegetable and/or of animal origin so as to bring about partial digestion of the egg-shell membrane material. Moreover, the finished products may contain active proteolytic enzymes which serve, in certain instances, to enhance the utility of the egg-shell membrane digest product.

Useful products can also be prepared from the egg-shell membrane mainly non-fibrous products herein described by oxidation and also by reduction techniques whereby to modify the disulfide linkages present in the egg-shell membrane substance. Oxidations have been carried out with hydrogen peroxide, urea peroxide, peracetic acid and performic acid to oxidize disulfide groups, present in egg-shell membrane materials, to intermediate states of oxidation of the disulfide linkages and to form cysteic acid groups. Such latter acidic groups can be reacted chemically for instance with basic amino acids such as lysine, arginine, histidine, and with methionine, as well as with basic peptides. Reductions have been carried out on the egg-shell membrane particulate mainly non-fibrous products described herein with mercaptoacetic acid, mercaptopropionic acid, substances which contain a reactive thiol group, and their soluble salts, to convert the disulfide groups present in the egg-shell membrane products to thiol groups. The resulting reduced egg-shell membrane products can then very readily be subjected to partial digestion with proteolytic enzymes to produce modified egg-shell membrane products useful for the treatment of denuded areas on the human body.

The dried egg-shell membrane mainly non-fibrous products, whether in sheet forms or in disaggregated particulate forms, can be sterilized, preferably prior to final packaging. This may be accomplished by techniques known in the art as, for example, by treatment with gaseous ethylene oxide or propylene oxide, or mixtures of each of these gases with other gases such as mixtures of ethylene oxide gas with carbon dioxide gas, preferably under pressure and at slightly elevated temperatures, for example at 40° C. to 75° C., and at pressures of 2 to 6 atmospheres for a period of 2 to 4 hours. Sterilization may also be carried out using beta propiolactone or, in certain cases, with moist or dry heat. However, depending upon the exact manner in which the aforesaid egg-shell membrane products have been prepared and handled, they may have a practical degree of sterility without the necessity for any special sterilizing treatments.

Further, it may be advantageous under certain circumstances to combine the dried egg-shell membrane mainly non-fibrous products, whether in sheet forms or in disaggregated particulate forms, with drugs, with enzymes,

with antibiotics and/or with bacteriostatic agents. To this end, said dried materials may be impregnated with the desired enzyme, drug, antibiotic or bacteriostatic substance or mixtures of these either by mechanical admixing of the said egg-shell membrane products with the drug in dry form or the drug may be dissolved in a suitable solvent and the drug solution applied to the egg-shell membrane products at convenient points in the preparation of the egg-shell membrane products herein described or to the finished products themselves. Typical of such antibiotics are neomycin and bacitracin, and particularly, broad spectrum antibiotics such as chlortetracycline, tetracycline, oxytetracycline, chloramphenicol, "sulfa" drugs and others.

While my invention is especially applicable to the treatment of egg-shell membranes obtained from chicken eggs, it is also useful in relation to egg-shell membranes obtained from eggs of other birds and fowl as, for instance, duck eggs. The examples listed above were carried out with egg-shell membranes prepared from the eggs of chickens because these represent the most available sources of large supplies of egg-shell membranes.

While I have described in detail processes of treating egg-shell membranes, it will be understood that other methods may be apparent to those skilled in the art.

What I claim as new and desire to protect by Letters Patent of the United States:

1. As an article of manufacture for assisting the healing of skin-denuded areas on the body, dry, finely divided pulverulent egg-shell membrane in essentially non-fibrous particle form.

2. An article of manufacture in accordance with claim 1, in which the egg-shell membrane particles are derived from uncooked egg-shell membranes.

3. As an article of manufacture for assisting the healing of skin-denuded areas on the body, dry, finely divided pulverulent egg-shell membrane in essentially non-fibrous form, the bulk density of said particles in the mass being from about 0.05 to 0.40 gram per cubic centimeter.

4. As an article of manufacture for assisting the healing of skin-denuded areas on the body, a dry felt fiber sheet containing a finely divided egg-shell membrane powder in essentially non-fibrous form.

5. As an article of manufacture for assisting the healing of skin-denuded areas on the body, a dry sheet comprising a layer of fiber felt material carrying finely divided egg-shell membrane powder in essentially non-fibrous form on a fabric base member.

References Cited by the Examiner

UNITED STATES PATENTS

2,039,312	5/36	Goldman	167—84
2,602,042	7/52	Abbott	167—65
2,833,669	5/58	Ziegler	167—84

OTHER REFERENCES

Harvey: Boston Medical and Surgical Journal, vol. 174, No. 18, pages 658 and 659, May 4, 1916.

Neuhauser: A.M.A. Archives of Dermatology, vol. 75, No. 3, pp. 401-404, March 1957.

JULIAN S. LEVITT, *Primary Examiner*.

FRANK CACCIAPAGLIA, JR., W. B. KNIGHT,
Examiners.

UNITED STATES PATENT OFFICE
CERTIFICATE OF CORRECTION

Patent No. 3,194,732

July 13, 1965

Irene Neuhauser

It is hereby certified that error appears in the above numbered patent requiring correction and that the said Letters Patent should read as corrected below.

Column 1, line 66, for "now-heated" read -- now-healed --; column 4, line 42, for "compirise" read -- comprise --; column 8, line 1, for "w./v." read -- W/V --; line 50, for "0.555" read -- 0.055 --; column 9, line 72, for "products" read -- product --; column 12, line 7, for "slovent" read -- solvent --.

Signed and sealed this 18th day of January 1966.

(SEAL)

Attest:

ERNEST W. SWIDER

Attesting Officer

EDWARD J. BRENNER

Commissioner of Patents