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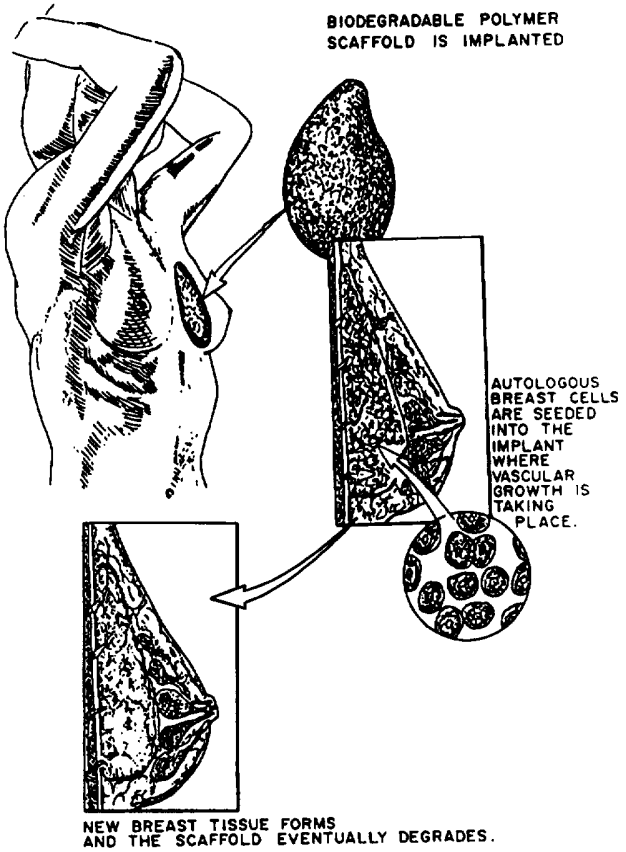
(54) Title: BREAST TISSUE ENGINEERING

(57) Abstract

Methods and compositions are described herein for reconstruction or augmentation of breast tissue. Dissociated cells, preferably muscle cells, are implanted in combination with a suitable biodegradable, polymeric matrix to form new tissue. There are two forms of matrices which can be used: a polymeric hydrogel formed of a material such as alginate having cells suspended therein, and a fibrous matrix having an interstitial spacing between about 100 and 300 microns. Preferred polymeric materials are those degrading over about one to two months, such as polylactic acid-glycolic acid copolymers. The matrices can be seeded prior to implantation or implanted, allowed to vascularize, then seeded with cells. In a preferred embodiment, the cell-matrix structures are implanted in combination with tissue expander devices. As cell-matrix is implanted, or cells proliferate and form new tissue, the expander size is decreased, until it can be removed and the desired reconstruction or augmentation is obtained. The preferred cell types are muscle cells, although other types of mesenchymal cells, fibroblasts, chondrocytes, and adipocytes can be used. Cells obtained from tissue such as the labia can be used for specialized applications such as formation of a nipple type tissue. Other materials, such as bioactive molecules that enhance vascularization of the implanted tissue and/or which inhibit ingrowth of fibrotic tissue, can be implanted with the matrix to enhance development of more normal tissue.



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BREAST TISSUE ENGINEERING

Background of the Invention

This invention is generally in the field of reconstruction and augmentation of breast tissue.

The breasts, or mammary glands, are modified sweat glands that are attached to the underlying muscle of the anterior chest wall by a layer of connective tissue. Internally, each mammary gland consists of 15-25 lobes, separated by dense connective tissue formed primarily by fibroblasts and bundles of collagen fibers, and adipose tissue containing adipose (fat) cells held together by reticular and collagen fibers. Within each lobe is a lactiferous duct that branches extensively. At the ends of the smallest branches are the glandular epithelial cells (alveolar cells) that synthesize and secrete milk into the duct system. The ducts, which are composed of simple cuboidal and columnar epithelium, and the alveolar cells are embedded in loose connective tissue containing collagen fibers and fibroblasts, lymphocytes, and plasma cells that secrete immunoglobulin A into the milk, conferring passive immunity on the newborn. Just external to the alveolar and duct epithelial cells are myoepithelial cells that respond to neural and hormonal stimuli by contracting and expressing the milk. Each lactiferous duct opens onto the surface of the breast through the skin covering the nipple.

Surgery of the breast can be broadly categorized as cosmetic and therapeutic. Cosmetic surgeries include augmentation, for example, using implants; reduction; and reconstruction. Therapeutic surgery, which is the primary treatment for most early cancers, includes radical surgery that may involve removal of the entire soft tissue anterior chest wall and lymph nodes and vessels

extending into the head and neck; lumpectomy, which may involve only a small portion of the breast; and laser surgery for destruction of small regions of tissue. Reconstructive surgery and the use of implants is frequently combined with radical breast surgery. The radical mastectomy involves removal of the breast, both the major and minor pectoralis muscles, and lymph nodes.

More than 250,000 reconstructive procedures are performed on the breast each year. Women afflicted with breast cancer, congenital defects or damage resulting from trauma have very few alternatives to reconstruction. Breast reconstruction is frequently used at the time or, or shortly after, mastectomy for cancer. Reconstructive procedures frequently involve moving vascularized skin flaps with underlying connective and adipose tissue from one region of the body, e.g., the buttocks or the abdominal region, to the breast region. Surgeons also use breast implants for reconstruction.

There are numerous surgical methods of breast reconstruction, including tissue expansion followed by silicone implantation, latissimus dorsi flap, pedicled transversus abdominis myocutaneous flap (TRAM), free TRAM flap, and free gluteal flap. Full reconstruction often requires numerous procedures in addition to the mastectomy and primary reconstruction. Procedures include tissue-expander exchange for permanent implant, nipple reconstruction, revision of reconstruction, and mastopexy/reduction.

Unfortunately, silicone prosthesis, which are used for reconstruction and augmentation, have caused numerous medical complications. It would be desirable to have an alternative material for implantation.

Even with reconstructive surgical methods that are currently in use, it is extremely difficult to achieve tissue that looks and feels normal, particularly when there has been extensive removal of associated muscle tissue.

It is therefore an object of the present invention to provide methods and compositions for reconstruction and augmentation of breast tissue.

It is a further object of the present invention to provide methods and materials to provide breast structure which is tissue, not foreign material such as silicone, and has the appearance of normal tissue.

Summary of the Invention

Methods and compositions are described herein for reconstruction or augmentation of breast tissue. Dissociated cells, preferably muscle cells, are implanted in combination with a suitable biodegradable, polymeric matrix to form new tissue. There are two forms of matrices which can be used: a polymeric hydrogel formed of a material such as alginate having cells suspended therein, and a fibrous matrix having an interstitial spacing between about 100 and 300 microns. Preferred polymeric materials are those degrading over about one to two months, such as polylactic acid-glycolic acid copolymers. The matrices can be seeded prior to implantation or implanted, allowed to vascularize, then seeded with cells. In a preferred embodiment, the cell-matrix structures are implanted in combination with tissue expander devices. As cell-matrix is implanted, or cells proliferate and form new tissue, the expander size is decreased, until it can be removed and the desired reconstruction or augmentation is obtained. The preferred cell types are muscle cells, although

other types of mesenchymal cells, fibroblasts, chondrocytes, and adipocytes can be used. Cells obtained from tissue such as the labia can be used for specialized applications such as formation of a nipple type tissue. Other materials, such as bioactive molecules that enhance vascularization of the implanted tissue and/or which inhibit ingrowth of fibrotic tissue, can be implanted with the matrix to enhance development of more normal tissue.

The cell-matrix structures can be implanted at the time of surgery to remove cancerous breast tissue, during subsequent reconstructive surgery, or over a period of time, for example, weekly, if serial injections of cell-hydrogel suspensions are used to create the new tissue.

Brief Description of the Drawings

Figure 1 is a schematic of the process for implantation of dissociated cells on a polymeric matrix into breast for breast tissue augmentation.

Figure 2 is a schematic of a fibrous plate implanted into breast tissue with struts to provide support of surrounding tissue and skin and allow new tissue to be formed within the strut following injection of a cell-hydrogel suspension.

Figures 3A, 3B and 3C are schematics of the serial injection of a cell-hydrogel suspension following implantation of a tissue expander, with the tissue expander being decreased in size each time the suspension is injected. Figure 3A is the tissue expander maximally expanded; Figure 3B is with fluid withdrawn from the expander to create a space between the abutting tissue and the expander, into which cell-polymer suspension is injected; and

Figure 3C is with the expander maximally deflated, with new tissue forming in the space occupied by much of the tissue expander expanded as in Figure 1.

Detailed Description of the Invention

I. Cells to Be Implanted

Cells to be implanted are dissociated using standard techniques such as digestion with a collagenase, trypsin or other protease solution. Preferred cell types are mesenchymal cells, especially smooth or skeletal muscle cells, myocytes (muscle stem cells), chondrocytes, adipocytes, fibromyoblasts, and ectodermal cells, including ductile and skin cells. In some cases it may also be desirable to include nerve cells. Cells can be normal or genetically engineered to provide additional or normal function.

Cells are preferably autologous cells, obtained by biopsy and expanded in culture, although cells from close relatives or other donors of the same species may be used with appropriate immunosuppression. Immunologically inert cells, such as embryonic cells, stem cells, and cells genetically engineered to avoid the need for immunosuppression can also be used. Methods and drugs for immunosuppression are known to those skilled in the art of transplantation. A preferred compound is cyclosporin using the recommended dosages.

In the preferred embodiment, skeletal or smooth muscle cells are obtained by biopsy and expanded in culture for subsequent implantation. Skeletal or smooth can be easily obtained through a biopsy anywhere in the body, for example, skeletal muscle biopsies can be obtained easily from the arm, forearm, or lower extremities, and smooth

muscle can be obtained from the area adjacent to the subcutaneous tissue throughout the body. To obtain either type of muscle, the area to be biopsied can be locally anesthetized with a small amount of lidocaine injected subcutaneously. Alternatively, a small patch of lidocaine jelly can be applied over the area to be biopsied and left in place for a period of 5 to 20 minutes, prior to obtaining biopsy specimen. The biopsy can be effortlessly obtained with the use of a biopsy needle, a rapid action needle which makes the procedure extremely simple and almost painless. With the addition of the anesthetic agent, the procedure would be entirely painless. This small biopsy core of either skeletal or smooth muscle can then be transferred to media consisting of phosphate buffered saline. The biopsy specimen is then transferred to the lab where the muscle can be grown utilizing the explant technique, wherein the muscle is divided into very pieces which are adhered to culture plate, and serum containing media is added. Alternatively, the muscle biopsy can be enzymatically digested with agents such as trypsin and the cells dispersed in a culture plate with any of the routinely used medias. After cell expansion within the culture plate, the cells can be easily passaged utilizing the usual technique until an adequate number of cells is achieved.

II. **Device Fabrication:**

Three principle types of matrices can be used to create new tissues or augment tissues. The term "bioerodible", or "biodegradable", as used herein refers to materials which are enzymatically or chemically degraded *in vivo* into simpler chemical species.

Hydrogel Polymer Solutions

In one embodiment, polymers that can form ionic hydrogels which are malleable are used to support the cells. Injecting a suspension of cells in a polymer solution may be performed to improve the reproducibility of cell seeding throughout a device, to protect the cells from shear forces or pressure induced necrosis, or to aid in defining the spatial location of cell delivery. The injectable polymer may also be utilized to deliver cells and promote the formation of new tissue without the use of any other matrix.

In a preferred embodiment, the hydrogel is produced by cross-linking the ionic salt of a polymer with ions, whose strength increases with either increasing concentrations of ions or polymer. The polymer solution is mixed with the cells to be implanted to form a suspension, which is then injected directly into a patient prior to hardening of the suspension. The suspension subsequently hardens over a short period of time due to the presence *in vivo* of physiological concentrations of ions such as calcium in the case where the polymer is a polysaccharide such as alginate.

Polymers

The polymeric material which is mixed with cells for implantation into the body should form a hydrogel. A hydrogel is defined as a substance formed when an organic polymer (natural or synthetic) is cross-linked via covalent, ionic, or hydrogen bonds to create a three-dimensional open-lattice structure which entraps water molecules to form a gel. Examples of materials which can be used to form a hydrogel include polysaccharides such as alginate, polyphosphazenes, and polyacrylates such as hydroxyethyl methacrylate

(HEMA), which are crosslinked ionically, or block copolymers such as Pluronics™ or Tetronics™, polyethylene oxide-polypropylene glycol block copolymers which are crosslinked by temperature or pH, respectively. Other materials include proteins such as fibrin, polymers such as polyvinylpyrrolidone, hyaluronic acid and collagen.

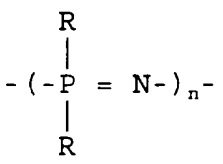
In general, these polymers are at least partially soluble in aqueous solutions, such as water, buffered salt solutions, or aqueous alcohol solutions, that have charged side groups, or a monovalent ionic salt thereof. Examples of polymers with acidic side groups that can be reacted with cations are poly(phosphazenes), poly(acrylic acids), poly(methacrylic acids), copolymers of acrylic acid and methacrylic acid, poly(vinyl acetate), and sulfonated polymers, such as sulfonated polystyrene. Copolymers having acidic side groups formed by reaction of acrylic or methacrylic acid and vinyl ether monomers or polymers can also be used. Examples of acidic groups are carboxylic acid groups, sulfonic acid groups, halogenated (preferably fluorinated) alcohol groups, phenolic OH groups, and acidic OH groups.

Examples of polymers with basic side groups that can be reacted with anions are poly(vinyl amines), poly(vinyl pyridine), poly(vinyl imidazole), and some imino substituted polyphosphazenes. The ammonium or quaternary salt of the polymers can also be formed from the backbone nitrogens or pendant imino groups. Examples of basic side groups are amino and imino groups.

Alginate can be ionically cross-linked with divalent cations, in water, at room temperature, to form a hydrogel matrix. Due to

these mild conditions, alginate has been the most commonly used polymer for hybridoma cell encapsulation, as described, for example, in U.S. Patent No. 4,352,883 to Lim. In the Lim process, an aqueous solution containing the biological materials to be encapsulated is suspended in a solution of a water soluble polymer, the suspension is formed into droplets which are configured into discrete microcapsules by contact with multivalent cations, then the surface of the microcapsules is crosslinked with polyamino acids to form a semipermeable membrane around the encapsulated materials.

Polyphosphazenes are polymers with backbones consisting of nitrogen and phosphorous separated by alternating single and double bonds. Each phosphorous atom is covalently bonded to two side chains ("R"). The repeat unit in polyphosphazenes has the general structure:



where n is an integer.

The polyphosphazenes suitable for cross-linking have a majority of side chain groups which are acidic and capable of forming salt bridges with di- or trivalent cations. Examples of preferred acidic side groups are carboxylic acid groups and sulfonic acid groups. Hydrolytically stable polyphosphazenes are formed of monomers having carboxylic acid side groups that are crosslinked by divalent or trivalent cations such as Ca^{2+} or Al^{3+}. Polymers can be synthesized that degrade by hydrolysis by incorporating monomers having imidazole, amino acid ester, or glycerol side groups. For example, a polyanionic poly[bis(carboxylatophenoxy)] phosphazene (PCPP)

can be synthesized, which is cross-linked with dissolved multivalent cations in aqueous media at room temperature or below to form hydrogel matrices.

Bioerodible polyphosphazenes have at least two differing types of side chains, acidic side groups capable of forming salt bridges with multivalent cations, and side groups that hydrolyze under *in vivo* conditions, e.g., imidazole groups, amino acid esters, glycerol and glucosyl. The term bioerodible or biodegrable, as used herein, means a polymer that dissolves or degrades within a period that is acceptable in the desired application (usually *in vivo* therapy), less than about five years and most preferably less than about one year, once exposed to a physiological solution of pH 6-8 having a temperature of between about 25°C and 38°C. Hydrolysis of the side chain results in erosion of the polymer. Examples of hydrolyzing side chains are unsubstituted and substituted imidizoles and amino acid esters in which the group is bonded to the phosphorous atom through an amino linkage (polyphosphazene polymers in which both R groups are attached in this manner are known as polyaminophosphazenes). For polyimidazolephosphazenes, some of the "R" groups on the polyphosphazene backbone are imidazole rings, attached to phosphorous in the backbone through a ring nitrogen atom. Other "R" groups can be organic residues that do not participate in hydrolysis, such as methyl phenoxy groups or other groups shown in the scientific paper of Allcock, et al., Macromolecule 10:824-830 (1977).

Methods for synthesis and the analysis of various types of polyphosphazenes are described by Allcock, H.R.; et al., Inorg. Chem. 11, 2584 (1972); Allcock, et al., Macromolecules 16, 715

(1983); Allcock, et al., Macromolecules 19, 1508 (1986); Allcock, et al., Biomaterials, 19, 500 (1988); Allcock, et al., Macromolecules 21, 1980 (1988); Allcock, et al., Inorg. Chem. 21(2), 515-521 (1982); Allcock, et al., Macromolecules 22, 75 (1989); U.S. Patent Nos. 4,440,921, 4,495,174 and 4,880,622 to Allcock, et al.; U.S. Patent No. 4,946,938 to Magill, et al.; and Grolleman, et al., J. Controlled Release 3, 143 (1986), the teachings of which are specifically incorporated herein by reference.

Methods for the synthesis of the other polymers described above are known to those skilled in the art. See, for example Concise Encyclopedia of Polymer Science and Polymeric Amines and Ammonium Salts, E. Goethals, editor (Pergamen Press, Elmsford, NY 1980). Many polymers, such as poly(acrylic acid), are commercially available.

The water soluble polymer with charged side groups is crosslinked by reacting the polymer with an aqueous solution containing multivalent ions of the opposite charge, either multivalent cations if the polymer has acidic side groups or multivalent anions if the polymer has basic side groups. The preferred cations for cross-linking of the polymers with acidic side groups to form a hydrogel are divalent and trivalent cations such as copper, calcium, aluminum, magnesium, strontium, barium, and tin, although di-, tri- or tetra-functional organic cations such as alkylammonium salts, e.g., R_3N^+ -\/\/\/-$^+NR_3$ can also be used. Aqueous solutions of the salts of these cations are added to the polymers to form soft, highly swollen hydrogels and membranes. The higher the concentration of cation, or the higher the valence, the greater the degree of cross-linking of the polymer. Concentrations from as low as 0.005 M

have been demonstrated to cross-link the polymer. Higher concentrations are limited by the solubility of the salt.

The preferred anions for cross-linking of the polymers to form a hydrogel are divalent and trivalent anions such as low molecular weight dicarboxylic acids, for example, terepthalic acid, sulfate ions and carbonate ions. Aqueous solutions of the salts of these anions are added to the polymers to form soft, highly swollen hydrogels and membranes, as described with respect to cations.

A variety of polycations can be used to complex and thereby stabilize the polymer hydrogel into a semi-permeable surface membrane. Examples of materials that can be used include polymers having basic reactive groups such as amine or imine groups, having a preferred molecular weight between 3,000 and 100,000, such as polyethylenimine and polylysine. These are commercially available. One polycation is poly(L-lysine), examples of synthetic polyamines are: polyethyleneimine, poly(vinylamine), and poly(allyl amine). There are also natural polycations such as the polysaccharide, chitosan.

Polyanions that can be used to form a semi-permeable membrane by reaction with basic surface groups on the polymer hydrogel include polymers and copolymers of acrylic acid, methacrylic acid, and other derivatives of acrylic acid, polymers with pendant SO_3H groups such as sulfonated polystyrene, and polystyrene with carboxylic acid groups.

Method for Making Cell Suspensions

The polymer is dissolved in an aqueous solution, preferably a 0.1 M potassium phosphate solution, at physiological pH, to a concentration forming a polymeric hydrogel, for example, for

alginate, of between 0.5 to 2% by weight, preferably 1%, alginate. The isolated cells are suspended in the polymer solution to a concentration of between 1 and 50 million cells/ml, most preferably between 10 and 20 million cells/ml.

Polymeric Matrix

Matrix Configuration

For an organ to be constructed, successfully implanted, and function, the matrices must have sufficient surface area and exposure to nutrients such that cellular growth and differentiation can occur prior to the ingrowth of blood vessels following implantation. The time required for successful implantation and growth of the cells within the matrix is greatly reduced if the area into which the matrix is implanted is prevascularized. After implantation, the configuration must allow for diffusion of nutrients and waste products and for continued blood vessel ingrowth as cell proliferation occurs.

The organization of the tissue may be regulated by the microstructure of the matrix. Specific pore sizes and structures may be utilized to control the pattern and extent of fibrovascular tissue ingrowth from the host, as well as the organization of the implanted cells. The surface geometry and chemistry of the matrix may be regulated to control the adhesion, organization, and function of implanted cells or host cells.

In the preferred embodiment, the matrix is formed of polymers having a fibrous structure which has sufficient interstitial spacing to allow for free diffusion of nutrients and gases to cells attached to the matrix surface. This spacing is typically in the range of 100 to 300 microns, although closer spacings can be used if the matrix is implanted, blood vessels allowed to infiltrate

the matrix, then the cells are seeded into the matrix. As used herein, "fibrous" includes one or more fibers that is entwined with itself, multiple fibers in a woven or non-woven mesh, and sponge like devices.

Cells can either be implanted after seeding onto a matrix or injected into a matrix already implanted at the desired site. The latter has the advantage that the matrix can be used to prevascularize the site. In this case, the design and construction of the scaffolding is of primary importance. The matrix should be a pliable, non-toxic, injectable porous template for vascular ingrowth. The pores should allow vascular ingrowth and the injection of cells such as muscle cells without damage to the cells or patient. These are generally interconnected pores in the range of between approximately 100 and 300 microns. The matrix should be shaped to maximize surface area, to allow adequate diffusion of nutrients and growth factors to the cells and to allow the ingrowth of new blood vessels and connective tissue. At the present time, a porous structure that is resistant to compression is preferred for implantation, prevascularization, followed by seeding.

In the embodiment where the matrix is prevascularized, it may be desirable to incorporate into the matrix means for dispersing the cells throughout the matrix, for example, using catheters which can be removed following seeding.

The overall, or external, matrix configuration is dependent on the tissue which is to reconstructed or augmented. In most cases, the cell-matrix structure will be similar to the silicone implants now used, which are essentially disks that deform due to gravity to form a teardrop shape. The shape can also be obtained using

struts, as described below, to impart resistance to mechanical forces and thereby yield the desired shape. The shape of the matrix *per se* will not be disk shaped, but will appear disk shaped when seeded with the cells to be implanted, or will create the outline of a disk or tear drop shape following implantation.

Polymers

Either natural or synthetic polymers can be used to form the matrix, although synthetic polymers are preferred for reproducibility and controlled release kinetics. Synthetic polymers that can be used include bioerodible polymers such as poly(lactide) (PLA), poly(glycolic acid) (PGA), poly(lactide-co-glycolide) (PLGA), poly(caprolactone), polycarbonates, polyamides, polyanhydrides, polyamino acids, polyortho esters, polyacetals, polycyanoacrylates and degradable polyurethanes, and non-erodible polymers such as polyacrylates, ethylene-vinyl acetate polymers and other acyl substituted cellulose acetates and derivatives thereof, non-erodible polyurethanes, polystyrenes, polyvinyl chloride, polyvinyl fluoride, poly(vinyl imidazole), chlorosulphonated polyolifins, polyethylene oxide, polyvinyl alcohol, teflon®, and nylon. Although non-degradable materials can be used to form the matrix or a portion of the matrix, they are not preferred. The preferred non-degradable material for implantation of a matrix which is prevascularized prior to implantation of dissociated cells is a polyvinyl alcohol sponge, or alkylation, and acylation derivatives thereof, including esters. A non-absorbable polyvinyl alcohol sponge is available commercially as Ivalon™, from Unipoint Industries. Methods for making this material are described in U.S. Patent Nos. 2,609,347 to Wilson; 2,653,917 to

Hammon, 2,659,935 to Hammon, 2,664,366 to Wilson, 2,664,367 to Wilson, and 2,846,407 to Wilson, the teachings of which are incorporated by reference herein. These materials are all commercially available.

Examples of natural polymers include proteins such as albumin, collagen, synthetic polyamino acids, and prolamines, and polysaccharides such as alginate, heparin, and other naturally occurring biodegradable polymers of sugar units.

PLA, PGA and PLA/PGA copolymers are particularly useful for forming the biodegradable matrices. PLA polymers are usually prepared from the cyclic esters of lactic acids. Both L(+) and D(-) forms of lactic acid can be used to prepare the PLA polymers, as well as the optically inactive DL-lactic acid mixture of D(-) and L(+) lactic acids. Methods of preparing polylactides are well documented in the patent literature. The following U.S. Patents, the teachings of which are hereby incorporated by reference, describe in detail suitable polylactides, their properties and their preparation: 1,995,970 to Dorough; 2,703,316 to Schneider; 2,758,987 to Salzberg; 2,951,828 to Zeile; 2,676,945 to Higgins; and 2,683,136; 3,531,561 to Trehu.

PGA is the homopolymer of glycolic acid (hydroxyacetic acid). In the conversion of glycolic acid to poly(glycolic acid), glycolic acid is initially reacted with itself to form the cyclic ester glycolide, which in the presence of heat and a catalyst is converted to a high molecular weight linear-chain polymer. PGA polymers and their properties are described in more detail in Cyanamid Research Develops World's First Synthetic

Absorbable Suture", Chemistry and Industry, 905 (1970).

The erosion of the matrix is related to the molecular weights of PLA, PGA or PLA/PGA. The higher molecular weights, weight average molecular weights of 90,000 or higher, result in polymer matrices which retain their structural integrity for longer periods of time; while lower molecular weights, weight average molecular weights of 30,000 or less, result in both slower release and shorter matrix lives. A preferred material is poly(lactide-co-glycolide) (50:50), which degrades in about six weeks following implantation (between one and two months).

All polymers for use in the matrix must meet the mechanical and biochemical parameters necessary to provide adequate support for the cells with subsequent growth and proliferation. The polymers can be characterized with respect to mechanical properties such as tensile strength using an Instron tester, for polymer molecular weight by gel permeation chromatography (GPC), glass transition temperature by differential scanning calorimetry (DSC) and bond structure by infrared (IR) spectroscopy, with respect to toxicology by initial screening tests involving Ames assays and *in vitro* teratogenicity assays, and implantation studies in animals for immunogenicity, inflammation, release and degradation studies.

Polymer Coatings

In some embodiments, attachment of the cells to the polymer is enhanced by coating the polymers with compounds such as basement membrane components, agar, agarose, gelatin, gum arabic, collagens types I, II, III, IV, and V, fibronectin, laminin, glycosaminoglycans, polyvinyl alcohol, mixtures thereof, and other hydrophilic and peptide

attachment materials known to those skilled in the art of cell culture. A preferred material for coating the polymeric matrix is polyvinyl alcohol or collagen.

Struts

In some embodiments it may be desirable to create additional structure using devices provided for support, referred to herein as "struts". These can be biodegradable or non-degradable polymers which are inserted to form a more defined shape than is obtained using the cell-matrices, especially the hydrogel-cell suspensions. An analogy can be made to a corset, with the struts acting as "stays" to push the surrounding tissue and skin up and away from the implanted cells. In a preferred embodiment, the struts are implanted prior to or at the time of implantation of the cell-matrix structure. The struts are formed of a polymeric material of the same type as can be used to form the matrix, as listed above, having sufficient strength to resist the necessary mechanical forces.

Tissue Expanders

Alternatively, or in addition, tissue expanders can be used to create additional space for implantation of cell-matrix structures. Tissue expanders are commercially available and routinely used for expansion of skin, for example, prior to plastic surgery, as reviewed by Cohen, J. Dermatol. Surg. Oncol. 19:614-615 (1993), Bennett and Hirt, J. Dermatol. Surg. Oncol. 19:1066-1073 (1993), Hammond, et al., Plastic and Reconstructive Surgery, 92(2):255-259 (1993), Walton and Brown, Annals of Plastic Surgery 30(2), 105-110 (February 1993), Kenna, et al., Annals of Plastic Surgery 32, 346-349 (1994), the teachings of which are incorporated herein. When skin is tensioned for

long periods of time, weeks to months, it responds by a very significant stretching. This is associated with metabolic activity and tissue growth. The generally accepted definition of a tissue expander is a device that resides beneath the surface of the skin which is used to stretch the skin. A spherical tissue expander is a multi-dimensional expander, typically applied by volumetrically expanding a subcutaneous space with an inflatable device. Alternatively, multiple bolus materials can be implanted and the device shrunk or replaced through removal of one or more of the bolus materials.

The use of tissue expanders in breast reconstruction are well understood (see, for example, Hammond, et al., 1993). Several different types of anatomically oriented or shaped expanders have been designed to give a more natural contour to the reconstructed breast. Devices are commercially available, for example, from McGhan Medical Corporation, Santa Barbara, CA, Dow Corning-Wright, Arlington, TN, and Mentor Corporation, Goleta, GA.

It is important to remove pressure from the implanted cells which can kill the cells. For example, in one preferred embodiment described in more detail below, a hydrogel-cell suspension is injected into the area where tissue is to be created. The space for injection of the cell-polymer suspension is created by implantation of a tissue expander prior to injection of the hydrogel-cell suspension. The tissue expander is inflated or expanded through implantation of a desired number of modules, to maximize the space and skin required for formation of tissue. As shown in detail in Figures 3A, 3B, and 3C, each time cell-matrix is injected, the tissue expander is deflated

or a module is removed to leave a space of an equivalent amount to the volume of cell-matrix injected. Once the space is essentially filled with new tissue or cell-matrix suspension, the tissue expander is removed, using in most cases a local anesthetic and minor incision.

Additives to Polymer Matrices

In some embodiments it may be desirable to add bioactive molecules to the cells. A variety of bioactive molecules can be delivered using the matrices described herein. These are referred to generically herein as "factors" or "bioactive factors".

In the preferred embodiment, the bioactive factors are growth factors, angiogenic factors, compounds selectively inhibiting ingrowth of fibroblast tissue such as antiinflammatories, and compounds selectively inhibiting growth and proliferation of transformed (cancerous) cells. These factors may be utilized to control the growth and function of implanted cells, the ingrowth of blood vessels into the forming tissue, and/or the deposition and organization of fibrous tissue around the implant.

Examples of growth factors include heparin binding growth factor (hbgf), transforming growth factor alpha or beta (TGFβ), alpha fibroblastic growth factor (FGF), epidermal growth factor (TGF), vascular endothelium growth factor (VEGF), some of which are also angiogenic factors. Other factors include hormones such as insulin, glucagon, and estrogen. In some embodiments it may be desirable to incorporate factors such as nerve growth factor (NGF) or muscle morphogenic factor (MMP).

Steroidal antiinflammatories can be used to decrease inflammation to the implanted matrix,

thereby decreasing the amount of fibroblast tissue growing into the matrix.

Where selective chemotherapeutic agents are available which do not inhibit growth of normal cells, such as antibody targeted chemotherapeutic agents, these can be incorporated into the matrix and used to inhibit any residual cancer cells remaining following a mastectomy.

These factors are known to those skilled in the art and are available commercially or described in the literature. *In vivo* dosages are calculated based on *in vitro* release studies in cell culture; an effective dosage is that dosage which increases cell proliferation or survival as compared with controls, as described in more detail in the following examples. Preferably, the bioactive factors are incorporated to between one and 30% by weight, although the factors can be incorporated to a weight percentage between 0.01 and 95 weight percentage.

Bioactive molecules can be incorporated into the matrix and released over time by diffusion and/or degradation of the matrix, they can be suspended with the cell suspension, they can be incorporated into microspheres which are suspended with the cells or attached to or incorporated within the matrix, or some combination thereof. Microspheres would typically be formed of materials similar to those forming the matrix, selected for their release properties rather than structural properties. Release properties can also be determined by the size and physical characteristics of the microspheres. Suitable microspheres and methods for their use in tissue generation is described in U.S. Serial No. 08/358,235 by David J. Mooney, Robert S. Langer, and Joseph P. Vacanti, entitled "Localized Delivery of Factors Enhancing

Survival of Transplanted Cells", co-filed herewith in the U.S. Patent and Trademark Office on December 16, 1994, the teachings of which are incorporated herein.

III. Methods for Implantation

As discussed generally above, there are three methods which can be used to create new breast tissue. These can be used alone or in various combinations. Variations include where the cell, which can be a hydrogel solution or a solid fibrous matrix, before implantation or serially introduced after matrix implantation to allow pre-vascularization of the matrix. The form of the engineered tissue can be regulated by utilizing a tissue expander to create the desired space for tissue formation, and then serially deflating the tissue expander while the cells of interest are delivered to this newly created space. This allows the tissue form to be pre-defined, and allows serial introduction of cells for form new tissue. Alternatively, a pre-formed matrix can be implanted, allowed to vascularize, then be seeded with dissociated cells which form new tissue, preferably as the matrix degrades. Selection of the appropriate system depends on the degree of augmentation required, and determines whether the whole injection can be performed at once, or alternatively, performed in a sequential manner, so as to allow for tissue formation with adequate vascularization before subsequent injections are performed.

Selection of cell type can be used to vary the texture of the implanted material, as well as the appearance. For example, cartilage can be used, if a more rigid implant is desired. In some embodiments it may be desirable to create softer

tissue, for example, using adipocytes or other soft tissue components.

Figure 1 is a schematic of the process for implantation of dissociated cells 10 on a polymeric matrix 12 into breast 14 for breast tissue augmentation. The cells attach to the matrix 12, which is originally disk shaped but deforms to a tear drop shape when implanted. As vascularization occurs and the matrix degrades, new tissue is formed.

Figure 2 is a schematic of a fibrous plate 20 implanted into breast tissue 22 with struts 24 to provide support of surrounding tissue and skin and allow new tissue to be formed within the strut following injection of a cell-hydrogel suspension (not shown).

Figures 3A, 3B and 3C are schematics of the serial injection of a cell-hydrogel suspension following implantation of a tissue expander (Figure 3A), with the tissue expander being decreased in size each time the suspension is injected (Figure 3B), so that new tissue forms in the space left as the expander is decreased in volume (Figure 3C).

We claim:

1. A method for augmentation or reconstruction of breast tissue comprising implanting an effective amount of dissociated cells selected from the group consisting of mesenchymal cells, myocytes, chondrocytes, adipocytes, fibromyoblasts, ectodermal cells, and nerve cells, to form breast tissue.

2. The method of claim 1 wherein the cells are smooth or skeletal muscle cells.

3. The method of claim 1 further comprising implanting the cells in combination with a matrix.

4. The method of claim 3 wherein the matrix is a biocompatible, biodegradable hydrogel.

5. The method of claim 3 wherein the matrix is implanted, allowed to vascularize, then seeded with cells.

6. The method of claim 3 wherein the matrix is a fibrous, polymeric matrix.

7. The method of claim 3 wherein the matrix is formed of a biodegradable polymer.

8. The method of claim 3 further comprising implanting struts with the matrix that support the surrounding tissue.

9. The method of claim 1 further comprising implanting bioactive molecules selected from the group of molecules enhancing vascularization, cell survival, proliferation or differentiation, inhibiting ingrowth of fibrotic tissue, inhibiting growth of cancerous cells, and inhibiting inflammation.

10. The method of claim 1 further comprising implanting a tissue expander prior to implanting cells in combination with a matrix, then implanting cells in combination with matrix each time the expander is decreased in size.

11. A composition for augmenting or reconstructing breast tissue comprising dissociated cells selected from the group consisting of mesenchymal cells, myocytes, chondrocytes, adipocytes, fibromyoblasts, ectodermal cells, and nerve cells in combination with a fibrous polymeric matrix, where the combination of the cells and matrix is effective to augment or reconstruct breast tissue.

12. The composition of claim 11 wherein the cells are smooth or skeletal muscle cells.

13. The composition of claim 11 wherein the fibrous matrix has a disk or tear drop shape.

14. The composition of claim 11 further comprising struts that support the surrounding tissue.

15. The composition of claim 11 further comprising bioactive molecules selected from the group of molecules enhancing vascularization, cell survival, proliferation or differentiation, inhibiting ingrowth of fibrotic tissue, inhibiting growth of cancerous cells, and inhibiting inflammation.

16. The composition of claim 11 further comprising a tissue expander.

17. The composition of claim 11 wherein the matrix is suitable for implantation and vascularization prior to seeding with cells, characterized by means for dispersing the cells throughout the matrix following implantation and being resistant to compression.

18. The composition of claim 11 wherein the matrix is formed of biodegradable polymers.

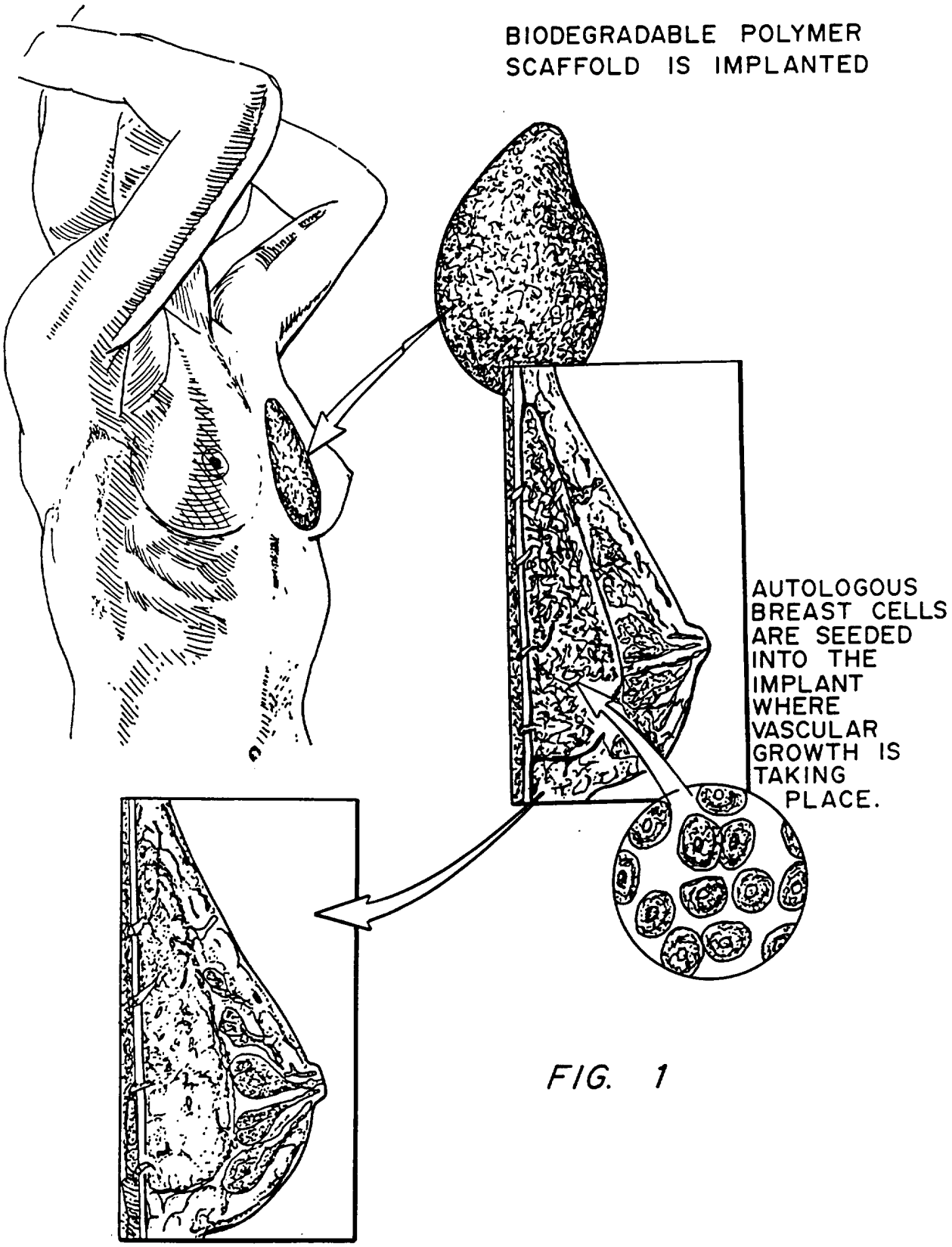

BIODEGRADABLE POLYMER SCAFFOLD IS IMPLANTED
AUTOLOGOUS BREAST CELLS ARE SEEDED INTO THE IMPLANT WHERE VASCULAR GROWTH IS TAKING PLACE.
NEW BREAST TISSUE FORMS AND THE SCAFFOLD EVENTUALLY DEGRADES.

FIG. 1

FIG. 2

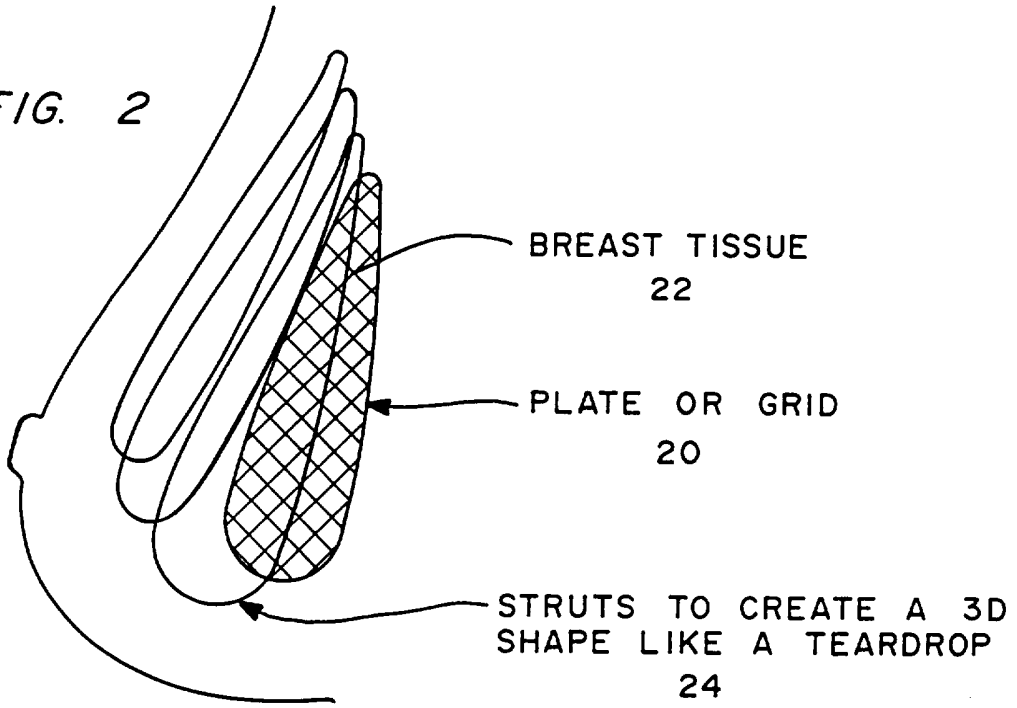
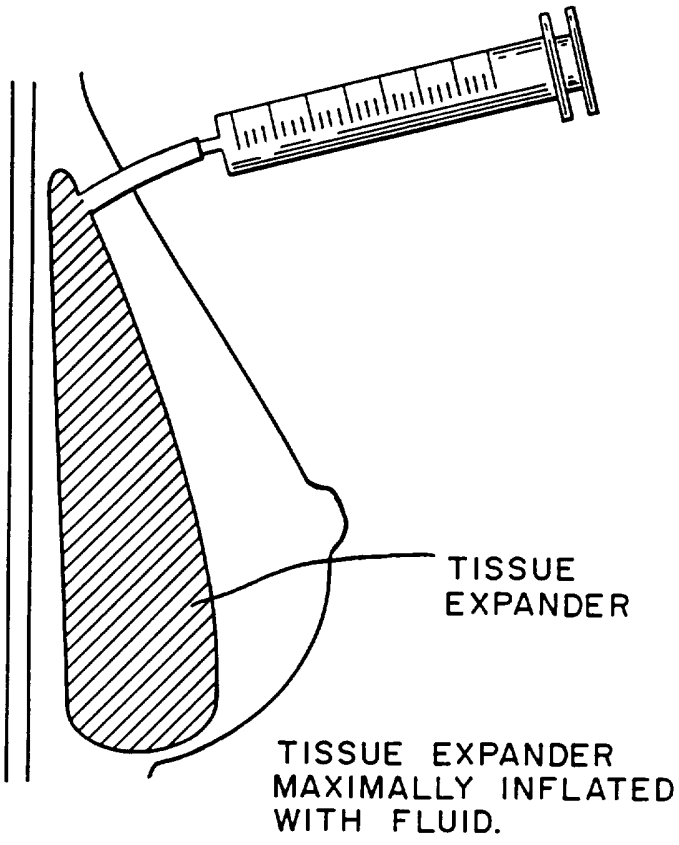

BREAST TISSUE
22
PLATE OR GRID
20
STRUTS TO CREATE A 3D
SHAPE LIKE A TEARDROP
24

FIG. 3a


TISSUE
EXPANDER
TISSUE EXPANDER
MAXIMALLY INFLATED
WITH FLUID.

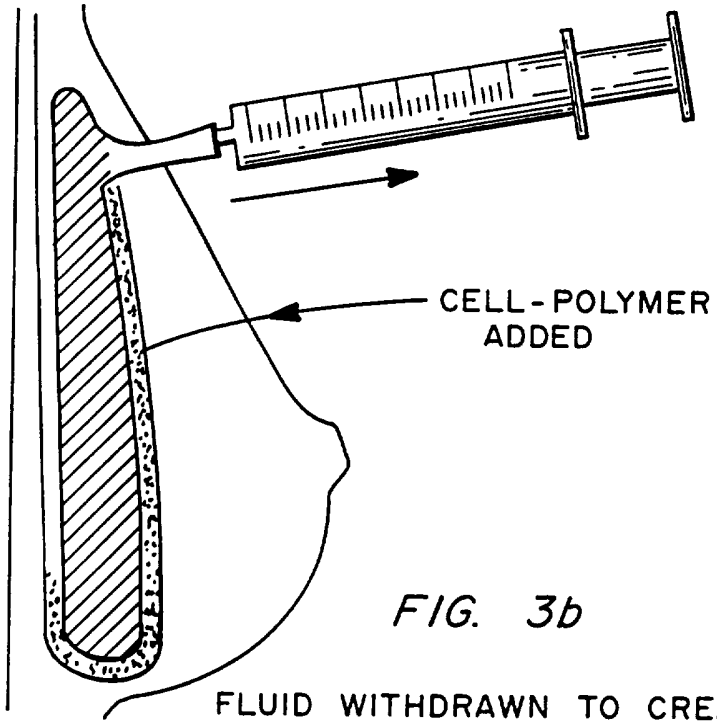


FIG. 3b

FLUID WITHDRAWN TO CREATE A
SPACE FOR CELL POLYMER INJECTION

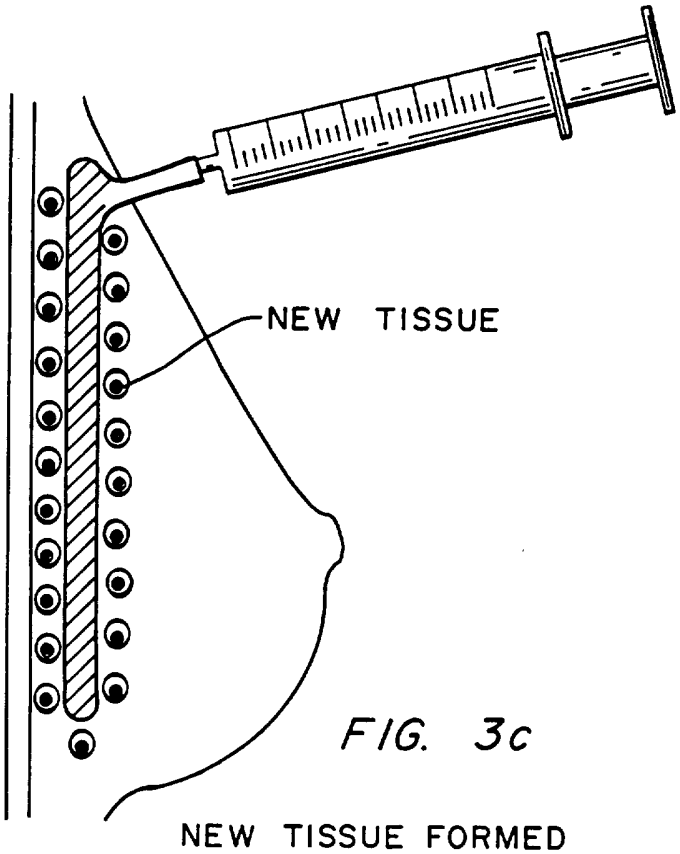


FIG. 3c

NEW TISSUE FORMED
IN SPACE

INTERNATIONAL SEARCH REPORT

Internatic ' Application No
PCT/US 95/16424

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61L27/00 C12N5/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,88 03785 (VACANTI J.) 2 June 1988 see claims 13,15-17,21,26	1-3,5-7, 9,11-13, 15,17,18
X	US,A,4 846 835 (GRANDE D.A.) 11 July 1989 see claims 1,2	1,3,4
X	WO,A,94 25079 (MASSACHUSETTS INSTITUTE OF TECHNOLOGY) 10 November 1994 see claims 1,9	1,3,5,7
X Y	WO,A,93 08850 (MASSACHUSSETTS INSTITUTE OF TECHNOLOGY) 13 May 1993 see claims 1,11,13,18	1-3,5,7, 9 6,11,12, 15,17,18

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
24 April 1996	20.05.96
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Authorized officer Peltre, C

Form PCT ISA 210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 95/16424

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO,A,93 07913 (CHILDREN'S MEDICAL CENTER) 29 April 1993 see claims 5,10,11,13 ---	6,11,12, 15,17,18
A	WO,A,92 07525 (BAXTER INTERNATIONAL) 14 May 1992 see claims 1,31 -----	1-18

Form PCT ISA 210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Interr onal application No.
PCT/US95/16424

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1-10
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 1 to 10 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

In .ation on patent family members

Internatior ' Application No
PCT/Us 95/16424

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-8803785	02-06-88	EP-A-	0299010	18-01-89
		JP-B-	7102130	08-11-95
		JP-T-	1501362	18-05-89
		US-A-	5041138	20-08-91
US-A-4846835	11-07-89	NONE		
WO-A-9425079	10-11-94	NONE		
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		JP-T-	7508663	28-09-95
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		AU-B-	8951491	26-05-92
		CA-A-	2070816	01-05-92
		CN-A-	1063046	29-07-92
		EP-A-	0507933	14-10-92
		JP-T-	5504704	22-07-93
		US-A-	5453278	26-09-95
		US-A-	5314471	24-05-94
		US-A-	5344454	06-09-94