Asian Biomedicine Vol. 5 No. 1 February 2011; 1-12 DOI: 10.5372/1905-7415.0501.001

Review article

Tissue engineering of bone and cartilage: a view through the patent literature

Lloyd Wolfinbarger Jr
Professor Emeritus, Old Dominion University, Norfolk, Virginia 23508 USA

Background: Tissue engineering takes on many approaches. It is mostly followed by those in the field through scientific literature. However, there is a virtually untapped resource in patent literature.
Objective: This review focuses on patents through the United States Patent and Trademark Office (USPTO). This source is used only because the author is most familiar with this resource. This article is not intended to be instructional regarding patents, patent law, or how to apply for patents, nor is it intended to be all inclusive of the patent literature. However, the reader might see the value of following the patent literature as a source of ideas, technologies, methodologies, and knowledge with respect to tissue engineering.

Keywords: Bioreactors, bone, cartilage, patent literature, tissue engineering

It is not possible to cover all the aspects of tissue engineering in a single review article. Therefore, only a few topics have been selected. Coverage of these selected topics will involve a summary review of relevant patents and patent applications found on the web site (*www.uspto.gov*) of the United States Patent and Trademark Office. Although there are many such web sites for many patent agencies around the world (*www.epo.org*), this review is limited to patent and patent applications available in the USPTO because the author is most familiar with this resource. The relevance of this approach to a review of tissue engineering lies in being able to illustrate a chronological sequencing over time for ideas (inventions), technology development, and understanding of issues important to this field.

It is important to state that a patent is not the same as a scientific publication when it comes to use as a reference in a scientific article. A patent is not intended to be science that is peer reviewed for its experimental design, statistical analysis of the data, accuracy of conclusions, or detailed descriptions of methodologies employed in collection of the data. Rather a patent is intended to claim a range or area over which the applicant wishes to have the ability to restrict use by others. Suffice it to say that a patent must describe a new and novel invention, must not have been obvious to one skilled in the art covered by that invention, and must describe the best methods/ways of practicing the invention, but there is rarely an intent in a patent to teach another how to practice the invention.

This brings the author to the next point of emphasis with respect to this review, which is how does some sequence of patent applications help to understand the evolution of specific tissue engineering technologies and how will these ideas eventually find their way into the scientific literature?

Typically, the evolution of a given technology involves the coming together of several inventions (as described in the patents) where the knowledge gained accumulates, resulting in the development of new ideas (inventions) that would not have been obvious without the accumulated knowledge.

Tissue engineering of bone

Whole human bones, pieces of human bones, and even components of human bones, for example bone collagens, are presently available for clinical use and are currently the "gold standard" for many clinical applications. Indeed, allograft bone is the most implanted bone material in the world and use of

Correspondence to: Lloyd Wolfinbarger Jr, PhD, BioScience Consultants, llc, 1509 Cedar Lane Norfolk, Virginia 23508, USA. E-mail: altmolec@yahoo.com

allograft bone products and materials are increasing each year. Opportunity to produce bone tissue or whole bone (or part of a whole bone) did not suddenly present itself. We might perhaps begin with US Patent number 7,494,811 that describes the "final product" and work backwards in the patent literature to see how this opportunity evolved [1]. The technology described in this patent involves the use of ground demineralized bone matrix (DBM) packed into a flow-through "column"-based bioreactor in association with a defined population of mammalian cells. The growth and differentiation factors present in the DBM will induce cells to become osteoblasts, i.e. bone tissue forming cells. These osteoblasts will synthesize and secrete proteinaceous and other polymeric macromolecules appropriate to the production of a mineralizable osteoid. These cell populations require a continuous supply of nutrients and a means of removing waste, this bioreactor system utilizes hollow-fiber technology that represents a capillary bed of blood vessels, which would typically be found in bone tissue. In principle, the DBM, cells, nutrient medium, and other additives are placed into the hollow fiber bioreactor. The nutrients facilitate cell growth and differentiation. The cells synthesize and lay down a proteinaceous matrix (an "osteoid" matrix). Depending on the desired final "product", these same cells can be encouraged to mineralize the matrix (or not). Stress and strain forces can be applied to the cells as the new matrix is being synthesized to simulate normal bone remodeling and a mineralized "load-bearing" bone graft (or a non-mineralized "partially load-bearing" bone graft) can be removed from the bioreactor for implantation or for decellularization to provide for an acellular base matrix, which the recipient can recellularize. The ability to enzymatically digest the hollow fibers from the final bone tissue (or bone) and incorporate angiogenic factors into the channels left by the hollow fibers prior to implantation was further described as an opportunity to promote vascular ingrowth into the bone tissue (or bone) following implantation of the tissue engineered construct.

This particular patented technology included four distinct technology areas whose use were not anticipated for the tissue engineering of bone tissues or of whole bones when they were initially conceived and patents applied for: 1) the ground demineralized bone matrix (DBM), 2) the hollow fiber based bioreactor, 3) the demineralization of ground bone to produce the DBM, and 4) the induction of cells to differentiate along an osteoprogenitor pathway (see **Fig. 1, 2** and **3**).

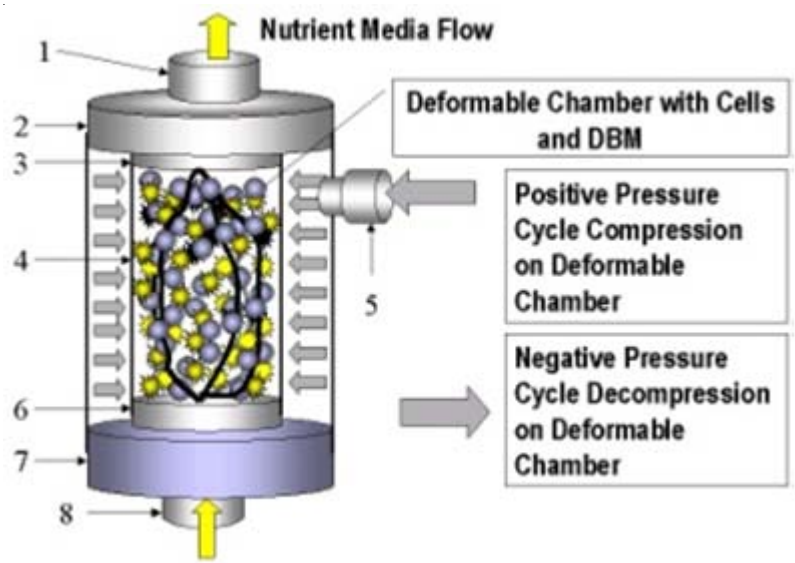

Fig. 1 An illustration of a typical bioreactor design showing inflow (**8**), outflow (**1**), deformable inner wall of the bioreactor (**4**), and an access port (**5**) permitting applications of positive and negative pressures to the deformable chamber containing cells and DBM.

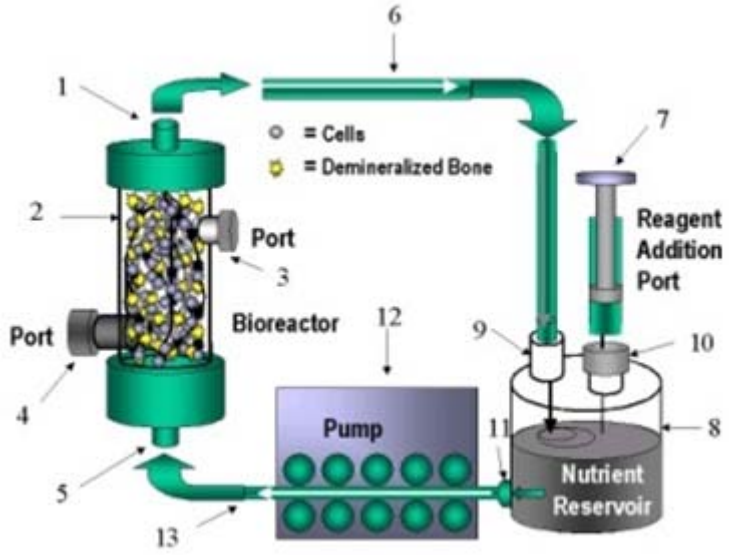

Fig. 2 An illustration of a typical bioreactor system showing use of a peristaltic pumping system (**12**) to distribute nutrient media from a nutrient media reservoir (removal of waste media is not shown in this diagram) through hollow fibers contained in the bioreactor. Additional reagents can be added over time via a reagent addition port (**10**).

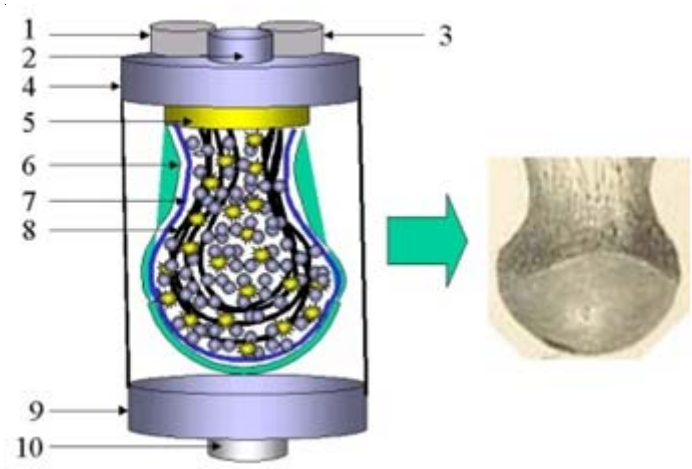

Fig. 3 A diagram to illustrate a different orientation of a bioreactor wherein the inlet and outlet ports (**1** and **3**, respectively) for the supply of nutrients and waste removal via the hollow fibers are on the same end of the bioreactor. The illustration is intended to describe the use of a bioreactor to incubate cells with DBM in a deformable inner chamber shaped to a configuration of a femur head (or humerus head).

Ground demineralized bone matrix (DBM)

That ground cortical bone could be demineralized and used to form new bone when implanted in animal model systems (for example mice or rats lacking a thymus, i.e. were athymic) was first recognized and described in an elegant patent with Marshall R. Urist as the inventor. It describes the demineralization of bone using acid solutions followed by solubilization of bone morphogenetic proteins (BMPs) by neutral salts such as urea and guanidine [2]. Examples used in this patent describe how such BMPs are examples of practical applications of BMPs when used in bone defects caused by injury, old infection, malignancy, and congenital defects. The process for the extraction of BMPs from bone and certain characteristics of those BMPs was further elaborated [3], and their use in compositions to induce bone formation in animals was disclosed [4]. The bioavailability of growth and

differentiation factors in bone as well as the observation that they could be "released" (made available to some cell population) during what would normally be a natural process of osteoclast cell-based demineralization preparatory to bone repair formed the basis for early suggestions that DBM could be made and used clinically to repair bone defects. That DBM could also be used *in vitro* to induce a cell population to differentiate along some osteoprogenitor pathway and that those induced cells might synthesize and secrete the molecular components of osteoid were of relevance to the invention [1].

Induction of cells to differentiate along some osteoprogenitor pathway

Given that the necessary growth and differentiation factors for induced cell differentiation could be easily provided by DBM in some *in vitro* process (analogous to its *in vivo* use in various clinical applications), it only became necessary to determine which cells might be most appropriate for use in some *in vitro* bioreactor based tissue engineering of bone tissue. One of the first patents to begin the discussion regarding the roles and possible uses of marrow-derived mesenchymal cells (i.e. mesenchymal stem cells) for the treatment of skeletal and other connective tissue disorders was US 5,226,914 [5]. Additional sources of "stem cells" for combinations into "shaped" implants for implantation into the body were later expanded to isolation of precursor cells from both hematopoietic and non-hematopoietic tissues [6]. Indeed, it was not even necessary to use "stem cells" for the induction of cells along some osteoprogenitor (osteoblastic) pathway by growth and differentiation factors. It appears that it is possible to use more differentiated cells, for example the common fibroblasts or periosteal cells, as cells to be induced by BMPs to participate in new bone formation both *in vivo* and *in vitro* [7] (**Fig. 4**).

There is a broad choice in the kinds of cells to be used in some bioreactor-mediated *in vitro g*rowth of "bone-like" biomaterials. Two patents described the use of *in vitro* cultures of cells to produce "produced matrix" for use in joint prostheses, maxillofacial implants, special surgery devices, or bone fillers [8], or to produce aggregates of cells onto biodegradable micro-carrier beads [9] that could be injected directly into the body or shaped for implantation into the body. Alternatively, the cells could be grown on microcarrier beads in a mold that was shaped to conform to the geometry of the desired body part to be replaced. These particular materials had to be used in clinical situations. They are used where either a ready supply of blood vessels to provide nutrients and carry away waste products was not critical to the functionality of the implanted materials or the amounts of such materials to be implanted were small in volume (cell densities) or in a site rich in rapidly exchanged body fluids.

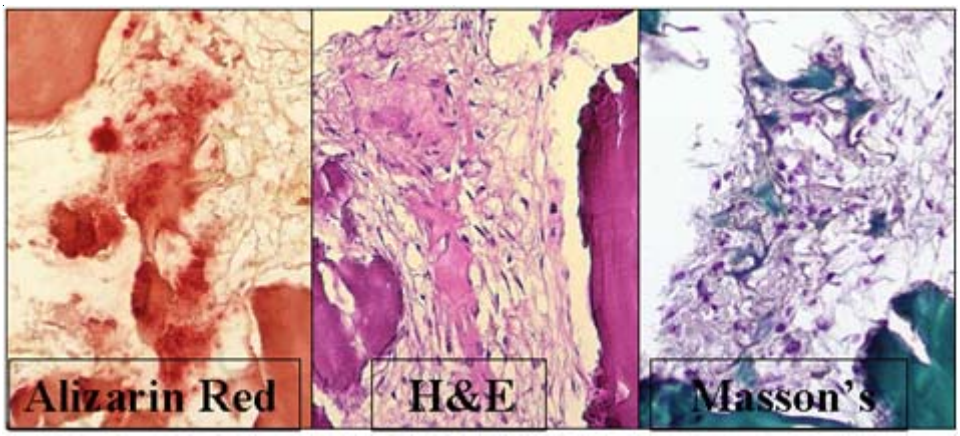

Fig. 4 Histology preparations showing cellular and matrix characteristics of non-mineralized (or at least not mineralized to the point of being similar to native bone tissue) DBM, cells, and formed base matrix (i.e. osteoid like material) from tissues grown in the bioreactor for three weeks. The Alizarin Red stain is intended to illustrate "mineralization", the H&E staining is intended to illustrate the cell population, and Masson's staining is intended to illustrate matrix characteristics.

Bioreactor technologies for in vitro growth of tissues

There is a strong and viable technology for the *in vitro* growth of cells in what is generally described as the BioProcessing Industry, i.e. the growth of cells in flow-through bioreactors for the production of vaccines, drugs, and other biologics used clinically. Such bioreactor technologies make use of "screw-type augers" installed coaxially in the cell culture compartment to provide a circulating flow of liquids. Cells being grown in such bioreactors are typically attached to microcarrier beads [10]. Such bioreactors have been described as having applications as organs. Presently, the use of *in vitro* cell culture to perform functions of tissues without actually becoming structurally similar to the organ whose functions are being mimicked has been illustrated [11].

Since most mammalian cells are "attachment dependent", it is important that cells being cultured in an *in vitro* bioreactor have something to physically attach to. This function is typically performed by the use of microcarrier beads that may be constructed of a variety of materials, for example acrylamide, polylactide/galactide polymers (PGLA), cellulosic, etc., or as in the case of the *in vitro* growth of bone (1), the DBM particles themselves (**Fig. 5**).

For bioreactors where stirring of the cells with microcarrier beads is not a viable option, high performance hollow fiber bioreactor technologies have been developed [12]. Such hollow fiber technology in a bioreactor allows for the high density culture of mammalian cells. In those, the density of the hollow fibers can be sufficiently concentrated that no cell is being cultured more than two millimeters from a hollow fiber (i.e. appropriate delivery of nutrients and removal of waste byproducts from that cell through a diffusional process). Without the hollow fibers within the bioreactor space, a high-density growth of cells would be hindered by a lack of nutrients, oxygen, and the removal of waste metabolic products and carbon dioxide. Such density of hollow fibers is illustrated diagrammatically in **Fig. 6.** The volumes immediately adjacent to hollow fibers are in "red" and cells outside this "red zone" might be expected to be nutrient starved and become hypoxic and perhaps even die and become necrotic.

DBM particles are equivalent to microcarrier beads. They are comprised of a collagenous matrix with a high surface area to volume ratio (typically each DBM particle lies within the particle size range of 125 to 710 (or less) mm and, by being irregular with respect to their surface area, present the opportunity for cells to occupy their surfaces in high densities). Indeed, much of the technologies [1] can be seen to have evolved from the successful use of *in vivo* bioassays designed to assess the new bone formation potential of DBM. In these *in vivo* bioassays, aliquots of DBM are implanted inter-muscularly in the upper hind legs of athymic mice (or rats) and after approximately 28 days, the implants are explanted and fixed for histological evaluation of new bone formation. If the DBM forms new bone in these *in vivo* conditions,

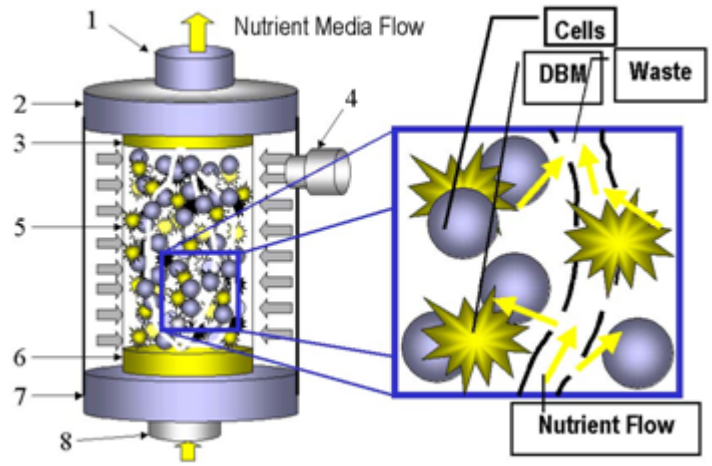

Fig. 5 This figure is designed to illustrate (right side of the diagram shows an enlargement of a small region of the DBM/cells/matrix present in a functioning bioreactor) the flow of nutrient materials through small pores in the hollow fiber walls such that the nutrients become available to the cells being stimulated to grow and differentiate by the growth and differentiation factors (i.e. bone morphogenetic proteins) present in the DBM.

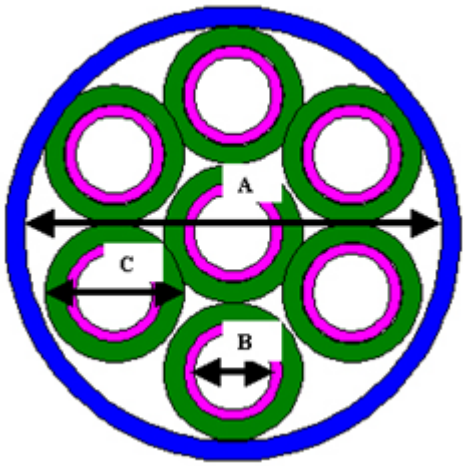

Fig. 6 Diagrammatic illustration of hollow fibers contained within a deformable inner wall of a bioreactor showing how it is important to maintain a sufficient hollow fiber "density" within a mixture of cells and DBM to be able to provide nutrients to the cells and carry away waste materials generated by the cells via some diffusion process. Cells much beyond about 2 mm from a hollow fiber can be expected to be nutrient deprived and perhaps hypoxic. "B" indicates the inside diameter of a hollow fiber. "C" indicates the relative marginal diameter wherein nutrient deliver and waste removal due to diffusion can be expected to be achieved. "D" indicates some necessary internal diameter appropriate for the deformable internal wall of the bioreactor.

there is the presumption that DBM would also form new bone (or at least bone-like) materials *in vitro* provided the culture conditions were appropriate to cellular growth, proliferation and differentiation, i.e. a hollow-fiber based bioreactor (an "artificial mouse") (**Fig. 7**).

Demineralization of ground bone to produce the DBM

We thus come to the last of the four technologies described earlier as being central to the *in vitro* growth of bone or bone-like tissues via a tissue engineering process. As was described by the patents by Urist [2-4], early efforts to demineralize ground bone in the production of DBM involved the use of multiple changes of a strong acid (for example hydrochloric acid at approximately 0.5 N) or the use of excess volumes of a strong acid in order to achieve what is typically referred to as "complete" demineralization of the ground bone particles. However, in 1991, a US patent application was filed, and Wolfinbarger et al. [13] described a method and means of monitoring the demineralization process. Subsequently, other patents [14] and [15] described a

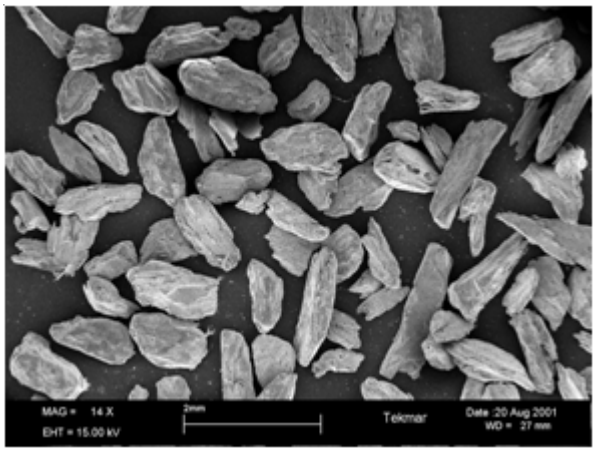

Fig. 7 Scanning electron photomicrograph of particles of ground demineralized bone. The ground demineralized bone particles shown fall in the particle size ranges of about 250 μm to 710 μm, however the figure is primarily provided to show particle geometry that determines packing geometry of such particles in a mixed bed of DBM and cells. Changing the particle size range-distribution and particle shape can be used to influence the processes of bone tissue (or bone) formation in a bioreactor over time.

means for DBM to be optimally "osteoinductive" (meaning to stimulate cells to differentiation along some osteoprogenitor pathway) and optimally active in forming new bone in some *in vivo* (nude mouse/ rat) bioassay. It was best to be able to stop the demineralization process at some demineralization time whereby enough mineral had been removed without damaging the biological properties of the produced DBM. Thus, by controlling the particle size distribution of the DBM it was possible to control the overall porosity of DBM materials and to control the average pore size available to cell synthesized matrix materials (spaces outside the demineralized bone particles) permitting the optimization of the osteoconductivity of the produced DBM. For example, DBM within a particle size range of 250 to 710 μm will occupy approximately 70% of the available volume when hydrated. The remaining 30% of the volume (the "void volume") will be that volume in which cells are free to fill with formed osteoid-like matrix. By controlling the particle size distribution (and particle shape) one can control this "void volume" (or porosity) as well as the physical dimensions (i.e. average pore size) outside the DBM particles. By controlling the demineralization process, it became possible to optimize the bioavailability of the growth and differentiation factors present in the bone tissue associated with the DBM particles and thus not overwhelm the cells with too much or too little growth and differentiation factors over the time of culture in a bioreactor.

Production of bone tissues and bones using tissue engineering:

The technology for *in vitro* growth of tissue suitable to bone formation is not in practical use at present [1] and it will probably be many years before it actually comes into use (if it ever does). It is quite one thing to invent something and quite another thing to bring it into practical applications. Such is the distinction between inventions and innovations. There are still a great many practical issues to be resolved and an even greater number of validation studies to demonstrate that bone tissue, or for that matter a whole bone no matter how small, can be produced for clinical applications. In addition, there is still the issue of whether or not to produce a non-mineralized bone tissue that will mineralize following implantation into the body. Should we produce a fully/partial load bearing fully/partially mineralized bone, produce bone tissue with the intended patients own cells, or produce bone with cells from another (allograft cells) and remove such cells via some decellularization process prior to clinical implantation?

Tissue engineering and cartilage repair

The repair of articular cartilage has been a clinical problem for as long as humankind has done something to load this tissue either too suddenly or with too much force over some period. Osteochondral defects represent a severe clinical problem today and a great many approaches have been undertaken to affect a repair to damaged articular cartilage. However, for purposes of this review, only a very select approach will be described and discussed. Thus we will discuss tissue engineering and use of osteochondral plugs to fill small defects.

By way of an introduction to this section, it is important to emphasize the view of the author with respect to articular cartilage. Although we normally discuss articular cartilage based on cells, glycosaminoglycans, hyaluronans, gross architecture, etc., the author would wish for you to focus on articular cartilage as a whole structure first. Articular cartilage covers the ends of load-bearing bones such that the ends of two bones might ride smoothly over each other's surfaces in the presence of loading. As a structure, articular cartilage is backed up by mineralized bone tissue, covered on its articulating surfaces by a smooth but very dense "membrane", and filled with high molecular weight collagens, proteoglycans and "water". When loads are applied to the articulating bones, the surfaces of the opposing articular cartilage structures press together, and the load is distributed throughout the articular cartilage as the areas (volumes) at the points of contact deform under the load. In that "water" is essentially not compressible, the only way the load can be distributed is for the "water" to move from under the load, pushing other molecules of "water" ahead of it. If there were no (or minimal) resistance to the movement of this "water" under some load, the articular cartilage would quickly deform until the dense "membranes" of each respective articular cartilage were pressed hard against the underlying bone structures. However, there is resistance to movement of "water" in articular cartilage and this resistance occurs at two primary (there are others, but let us focus on two) levels. One level involves the nature of the interlocked proteoglycans (and other high molecular weight molecules such as

collagens) whereby the density of these high molecular weight polymers physically impedes the movement of "water" within the articular cartilage, i.e. this inner fluid filled part of the articular cartilage is very viscous. A second level involves the chemical nature of the glycosaminoglycans (GAGs) that form an important component (molecular part) of the proteoglycans. These GAGs are typically shown in hand drawn (or electron photomicrographs) as "brushes" whereby the GAGs are shown as radiating out perpendicularly from the backbone of the protein part of the proteoglycan. Looking at the molecular structure of the GAGs, one quickly notes that each strand of the "brush" is a linear polymer of glycans where some glycans are sulfated (carry net negative charges at physiologic pH) and some glycans are aminated (carry net positive charges at physiologic pH). By balancing the ratios of negative and positive groups on the glycans, it is possible to vary the packing density of the individual "strands" of the "brush" part of the proteoglycan (the GAGs). If one minimizes the numbers of positively charged groups on the glycans, the overall negative charge distribution will tend to force the "strands" of the "brush" apart under normal conditions with the attendant filling of the space with "water" molecules. With application of a load to the articular cartilage structure, that physical loading can compress the molecular structure of the "brush" part of the proteoglycan literally squeezing the "water" from between the individual "strands" of the "brush" and into the bulk viscous material where further movement of the "water" is impeded as described above. When the loading is removed, the strong negative charges associated with the negatively charged glycans will cause the individual strands to move away from each other and the "brush" part of the proteoglycan will decompress leaving room for "water" to move back between the individual strands, i.e. move from the bulk fluid volume to the molecular fluid volume of the viscous material.

The point of this very general discussion on the nature of articular cartilage is to emphasize that by its very nature, articular cartilage is a fluid filled structure whose function in load-bearing is to control the movement of fluid (what the author has been referring to as "water") such that the load(s) is (are) dispersed throughout the articular cartilage "organ" and ultimately to the underlying bone. The primary role (in addition to being smooth) of the dense membrane comprising the articulating surface of the articular cartilage "organ" is to contain that fluid. Consider an example of a balloon filled with water. If one places his/her hand on the top of that water filled balloon and presses down, the balloon simply bulges outwards around the circumferential areas of that balloon and when the hand is removed from the top of the water filled balloon, it returns to its original "round" structure. If the membrane surrounding the balloon were less deformable, greater loads could be applied without the balloon bulging outwards and perhaps rupturing and if the water in that balloon were very viscous, the "bulging" and "returning to its original round structure" would occur more slowly. If you poke a hole in the membrane surrounding the water-filled balloon and then apply a load, the water simply flows out of the balloon and when the load is removed, the balloon does not return to its original shape.

We thus have the critical component of articular cartilage repair that represents the primary focus of this review of tissue engineering of osteochondral grafts for repair of articular cartilage. It is not enough to repair a defect in articular cartilage by filling in the defect with an osteochondral plug or with chondrocytes (or any other types of cell). The repair effort must repair the fluid management aspects of the complete articular cartilage "organ" or function of the articular cartilage will not be restored and the articular cartilage will continue to deteriorate.

This brings us to a series of pending US patent applications that attempt to cover the breadth of technologies necessary to the repair of articular cartilage. For this description of inventions and innovations that have been developed and used in attempts to affect a repair to damaged articular cartilage, we will take our discussion from the oldest to the newest (a reverse strategy to the description of the *in vitro* growth of bone tissues and bone). However, let me once again emphasize that due to the restricted nature of a review article, we will focus only on a limited number of technologies for the repair of focal defects on the load bearing aspects of articular cartilage.

In the repair of focal defect sites in articular cartilage, there are two main strategies. One strategy has been to debride the defect site and place populations of cells (with or without some matrix carrier) into that debrided defect site, seal the site with a "membrane" of some sort to restrict the cells to the site of implantation and expect that the cells will produce new cartilageous matrix and repair the

defect site. A second strategy has been to take an osteochondral (autograft or allograft) plug and use it to fill in a hole cut encompassing the defect, again with the expectation that the autologous/allogenous plug will work to absorb applied loads while the osteochondral plug is incorporating into the cartilage structure.

The first strategy, that of filling a defect site with cells is best exemplified by a commercially available technology known as Carticel® (Carticel is a registered trademark of Genzyme Corporation). The technology was initially described in 1998 and was eventually commercialized under the trade name of Carticel [16]. This patent provided methods and compositions for the repair of articular cartilage defects that involved the proliferation of "denuded chondrogenic cells", the seeding of these cells (plus synthesized matrix) into "pre-shaped wells" for further differentiation and synthesis of matrix, with eventual surgical implantation of these synthetic cartilage constructs into "predetermined sites" (articular cartilage) for integration. A unique aspect of this technology involved the use of autogenous cartilage from the intended recipient of the engineered cartilage as a source of the chondrogenic cells. Greater definition of this technology was later described [17] where the use of polypeptide growth factors, sources of the chondrogenic cells, addition of ascorbate to the cells as cultured in "step c", and perhaps more importantly the technology of preparing a "multi cell-layered synthetic cartilage patch" to the process were added. Retention of the engineered cartilage patch in the created implant site involved use of bioadhesives and an overlayered "periosteal" patch sutured to the recipients tissue. The importance of this technology was that it represented one of the earliest inventions falling under the auspices of tissue engineering that was successfully commercialized. The technology allowed the isolated chondrogenic cells to synthesize their own matrix and the chondrocyte/matrix "plug" was surgically transplanted into the defect site created by cutting out damaged cartilage. Thus, the technology was also important in that it introduced the concept of surgically excising a defect site in articular cartilage and replacing it with a "plug" of newly synthesized "cartilage material" with the expectation of regeneration of a fully functional articular cartilage structure-what we now describe as regenerative biology.

While the Carticel technology focused on the chondrogenic cells synthesizing the matrix that would eventually allow for the implantation of a "plug" graft, this concept has evolved over time such that other groups chose to produce a "biocompatible, resorbable type II collagen-based matrix" using solubilized animal cartilage tissue for use in culturing and growing cells (such as chondrocytes) [18]. In this particular technology, the approach involved the creation of a matrix, which was further cross-linked using a bifunctional crosslinking agent, which is "cyanidanol". Cells were then attached to this matrix prior to transplantion into a created defect site in the articular cartilage structure. Similarly, this particular technology [19] described the use of cells grown on microcarrier beads where the microcarrier material comprised various polysaccharides (for example arabinogalactan, dextran, pullulan, and amylose) crosslinked by a polyamine (for example lysine, gelatin, albumin).

Along this line of technology development for the repair of articular cartilage defects, the invention of a composition comprised "a cartilage-growth enhancing material containing minced juvenile cartilage particles and a matrix" was described [20]. These juvenile cartilage particles included dimensions of between one and 27 mm^3, and could be obtained from donors as old as "less than fifteen years of age" to as young as "prepubescent". Similar to prior technologies along this strategy, a "retainer" was used to keep the implanted materials at the implant site and these retainers could include "periosteal flaps", synthetic membranes, etc., however this retainer could comprise a "sutureless attachment".

The second strategy, involves obtaining an osteochondral plug from areas of the autogenous articular cartilage structure not directly involved in the transmittal of loads across two bones as well as that of obtaining an osteochondral plug from an allogenous articular cartilage structure. This strategy has also been successfully commercialized. At present, there are issues of whether or not it is essential to use such osteochondral plugs retaining viable cells. However, this issue will be left to others. This review will simply focus on the evolution of technologies centered on the use of osteochondral plugs as a means of filling a created surgical site that removed the articular cartilage defect. This strategy is different from the aforementioned strategy in that it involves the use of native articular cartilage tissues to fill a defect site in the articular cartilage. These native articular cartilage

tissues are expected to either integrate into the defect area via the normal biosynthetic activities of viable chondrocytes present in the "vital" (autograft or allograft) cartilage tissue or to integrate into the defect area by way of recipient cells migrating into the "non-vital" (mostly allograft) cartilage tissue and assuming the role of integrating the implanted tissue into the recipients tissue structure.

It is the nature of these osteochondral plugs and how they are surgically implanted (transplanted) into the created defect site in the patient that has become important to efforts of inventing and innovating. It is also this nature of how the osteochondral plugs are implanted using either strategy one or two that are critical to the eventual clinical outcomes of articular cartilage repair.

The repair of damaged articular cartilage has been described previously [21]. The plugs may have articular surfaces formed on "either end", but in each instance the process involves the removal of defective cartilage to create a recipient "socket" for the implant. The implant is sized to fit the recipient socket and the implants are preferably formed of a hydrogen material such as Salubrin□. Metal or allograft implants can also be used. A described application of this osteochondral plug involves matching the curvature of the implanted osteochondral plug to the curvature of the recipient articular cartilage.

Another patent evolves the concept of an osteochondral plug for use in repairing a defect site in articular cartilage by providing for a "cartilage repair assembly comprising a cylindrically shaped allograft structure of subchondral bone with an integral overlying smaller diameter cartilage cap" [22]. The "cap" is treated to remove cellular debris and proteoglycans. However, one essential element of this technology involves the creation of a shaped structure that will dimensionally fit into a drilled bone. This is done so that the subchondral bone of the structure engages the sidewall of the bone portion of the drilled bore in an interference fit while the cartilage cap is spaced from the cartilage portion of the sidewall of the drilled bore, forming a gap in which a milled cartilage and biocompatible carrier mixture can be placed. The milled cartilage and biocompatible carrier are directed at allowing cell transfer throughout the defect area. This technology is further expanded on in another patent [23]. The implant includes a three-dimensional body formed of cancellous bone having a demineralized section and a cartilage layer formed on a surface of the demineralized section. The cartilage layer is formed by a method of isolating chondrocytes, cultivating the chondrocytes to expand their numbers, and suspending the cultured chondrocytes in agarose where they form a plurality of layers of chondrocytes on the demineralized section of the implant. As an update on this technology, a new US patent application [24] further describes additional attributes to engineered osteochondral construct(s) for treatment of articular cartilage defects.

Therefore, we come to the "final" aspects of this tissue engineering technology development with respect to this review by describing technologies that have been invented, but are yet to be "innovated" (i.e. "commercialized" by being made available for clinical use). Four US patent applications have been published describing the preparation and implantation of osteochondral plugs into created articular cartilage defect sites. The production of osteochondral plugs was described in a patent published in 2008 [25], and is applicable to tissues that are cleaned of bone marrow and cellular materials in the bone parts of the plug, decellularized in the cartilage parts of the plug, and disinfected in both the bone and cartilage parts of the plug. A second patent application [26] described how various osteochondral plugs were prepared (shaped, perforated, etc.) such that opportunities for cellular infiltration and control of rotation of the graft in an implant site, and cutting and milling of the graft were improved. A third patent application [27] described processes for removing donor cells from the cartilage portion of a plug graft and for recellularizing the bone and/or cartilage portions of the plug graft using bioreactor technologies. The fourth patent application [28] is the technological approach that differentiates this technology from previous allograft/autograft osteochondral plug based repair(s) of osteochondral defects. The claims in this patent application are directed at affecting a tight fit (as opposed to a gap between donor and recipient tissues to be filled with materials (23)) between the donor and recipient tissues. This is done to allow creation of molecular bonds between the circumferential edges of the implanted osteochondral plug and the circumferential (inner) edges of the created osteochondral defect site. The objective of these molecular bonds is to create a barrier to the movement of fluids from the cartilage tissue into the synovial fluid volume and from the synovial fluid volume into the cartilage tissue when loads are applied to the articular cartilage. It is this restriction of movement of fluids (water-based solutions) around an implanted osteochondral plug that are claimed to be essential to the successful outcomes of articular cartilage repair (**Fig. 8**).

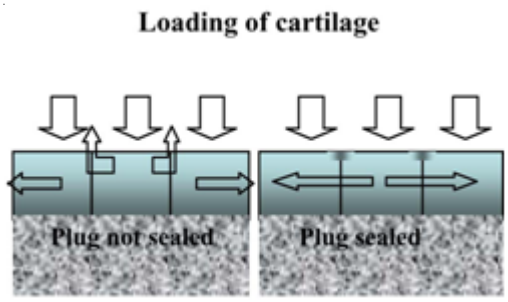

Fig. 8 Cartoon to illustrate relative movements of articular cartilage fluids ("water") when loads are applied to the articular cartilage into which an osteochondral plug has been inserted in a created defect site. Diagram on the left shows fluids leaking into the intra-articular spaces with applications of loading whereas diagram on the right shows fluids moving only within the articular cartilage with applications of loading.

The arguments put forth for the efficacy of this approach are that repair of articular cartilage pathologies is less about the tissue and more about the overall structure of that tissue. With articular cartilage (osteochondral grafts), researchers and clinicians will eventually understand that cartilage is less of a tissue to be repaired with "patches" (osteochondral plugs) than it is an "organ" with functions to be maintained. Articular cartilage is a fluid filled structure designed to absorb loads by restricting the movement of those fluids present within that fluid filled structure. Breaking the "seal" (the tough "membrane" surrounding the articular cartilage) whether it be by injury or surgery causes the loss of hydraulic function (restricted movement of fluids) and repairing the defect is less about filling in some "hole" than it is about restoring the nature of the fluid filled structure by restoring hydraulic function (sealing the "seal"). Until we understand the nature of the articular cartilage we are attempting to restore function to, we cannot hope to process articular cartilage grafts by any method and use them to fill holes in a non-functioning "organ".

Concluding remarks

We come to an end of this brief overview of how information present within the patent literature can provide significant insight into the development of new technologies involving tissue engineering that is based on sound science. The author has deliberately chosen to not cite published scientific literature in numerous scientific journals feeling that the reader would have already read much of this information. It should once again be emphasized that the patent literature is directed at describing science and technologies in ways that would perhaps be less appropriate to the traditional scientific literature. However, the patent literature is nonetheless an important window into why we strive to conduct good scientific studies and to report the results of those studies to our peers and colleagues.

Acknowledgements

The author retired as a University Professor after 30 years, and is now Professor Emeritus at Old Dominion University. The author is a consultant of a number of medical and biomedical companies and has no commercial or economic interest(s) that might permit him to derive financial benefits from this review article. The author thanks Dr. Sittisak Honsawek, Faculty of Medicine of Chulalongkorn University for his generous invitation to visit his department, which led to the idea for this review article.

References

1. Wolfinbarger L Jr, Qin X. 2009. In vitro growth of tissue suitable to the formation of bone and bone forming tissue formed thereby. US Patent 7,494,811. February 24, 2009.
2. Urist M R. Bone morphogenetic protein process. US Patent 4,294,753. October 13, 1981.
3. Urist MR. Bone morphogenetic protein. US Patent 4,455,256. June 19, 1984.
4. Urist MR. Bone morphogenetic protein composition. US Patent 4,619,989. October 28, 1986.
5. Caplan AI, Stephen EH. Method for treating connective tissue disorders. US Patent 5,226,914. July 13, 1993.
6. Peterson DR, Nousek-Goebl N. Isolation of precursor cells from hematopoietic tissues and their use in vivo bone and cartilage regeneration. US Patent 6,200,606. March 13, 2001.
7. Breitbart AS, Grande DAD. 1997. Tissue-engineered

bone repair using cultured periosteal cells. US Patent 5,700,289. December 23, 1997.
8. Van Blitterswijk CA, De Bruijn JD, Bovell YP. Method for in vitro production of bone. US Patent 6,152,964. November 28, 2000.
9. Frondoza CG, Fink DJ, Hungerford DS, Shikani AH. Method for fabricating cell-containing implants. US Patent 6,886,568. May 3, 2005.
10. Armstrong DW, Fleming P, Grenzowski DG. Cell culture bioreactor. US Patent 4,906,577. March 6, 1990.
11. Hu W-S, Cerra FB, Nyberg SL, Scholz MT, Shatford RA. Bioreactor device with application as a bioartificial liver. US. Patent 5,605,835. February 25, 1997.
12. Goffe RA. High performance cell culture bioreactor and method. US Patent 5,622,857. April 22, 1997.
13. Wolfinbarger L Jr, Bottenfield S. Process for demineralization of bone using column extraction. US Patent 5,275,954. January 4, 1994.
14. Wolfinbarger L Jr. Process for producing osteoinductive bone and osteoinductive bone produced thereby. US Patent 6,189,537. February 20, 2001.
15. Wolfinbarger L Jr. Process for producing osteoinductive bone and osteoinductive bone produced thereby. US Patent 6,305,379. October 23, 2001.
16. Tubo RA, Barone LM, Wrenn CA. Methods and compositions for the repair of articular cartilage defects in mammals. US Patent 5,723,331. March 3, 1998.
17. Tubo RA, Barone LM, Wrenn CA. Methods for the repair of articular cartilage defects in mammals. US. Patent 5,786,217. July 28, 1998.
18. Muller PK, Behrens P, Giannetti B, Rohwedel J, Russlies M. Cellular matrix. US Patent 6,623,963. September 23, 2003.
19. Hungerford DS, Frondoza CG, Sohrabi A, Shikani AH, Domb AJ. Cell-culture and polymer constructs. US Patent 6,378,527. April 30, 2002.
20. Kizer N, Spiro R, Yao JQ, Blanchard CR. Particulate cartilage system. US Patent 7,824,711. November 2, 2010.
21. Schmieding R. Method and instrumentation for osteochondral repair using preformed implants. US Patent 7,264,634. September 4, 2007.
22. Truncale KG, Sunwoo MH, Gertzman AA, Tomford WW, Yannariello-Brown JL. Cartilage allograft plug. US Patent 7,488,348. February 10, 2009.
23. Syring C, von Versen RWA. Implant for articular cartilage repair. US Patent 7,815,926. October 19, 2010.
24. Syring C, von Versen RWA. US Patent Application 2010/0241228. Published September 23, 2010.
25. Chen SS, Qin X, Chen J, Wolfinbarger L Jr. Cleaning and devitalization of cartilage. US Patent Application 2008/0077251. Published March 27, 2008.
26. Chen SS, Qin X, Chen J, Wolfinbarger L Jr. Crafting of cartilage. US Patent Application 2009/0024223. Published January 22, 2009.
27. Chen SS, Qin X, Chen J, Wolfinbarger L Jr, Moore M, Thomas R, Jones AL. Devitalization and recellularization of cartilage. US Patent Application 2009/0024229. Published January 22, 2009.
28. Chen SS, Qin X, Chen J, Wolfinbarger L Jr. Implantation of cartilage. US Patent Application 2009/0024224. Published January 22, 2009.