# Biomaterials and Tissue Engineering for Regenerative Repair of Articular Cartilage Defects

## *Eklem Kıkırdağı Hasarlarının Yenilenme ile Onarılmasında Biyomalzemeler ve Doku Mühendisliği*

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**Abstract**

Articular cartilage defects heal very poorly and lead to degenerative arthritis. Existing medications cannot promote healing process; cartilage defects eventually require surgical replacements with autografts. As there is not enough source of articular cartilage that can be donated for autografting, materials that promote cartilage regeneration are important in both research and clinical applications. Tissue engineering involves cell growth on biomaterial scaffolds in vitro. These cells are then injected into cartilage defects for biological in vivo regeneration of the cartilage tissue. This review aims first to provide a brief introduction to the types of materials in medicine (biomaterials), to their roles in treatment of diseases, and to design factors and general requirements of biomaterials. Then, it attempts to sum up the recent advances in engineering articular cartilage; one of the most challenging area of study in biomaterials based tissue engineering, as an example to the research on regenerative solutions to musculoskeletal problems with an emphasis on the biomaterials that have been developed as scaffolds for cartilage tissue engineering. The definitive goal on cartilage regeneration is to develop a system using biomimetic approach to produce cartilage tissue that mimics native tissue properties, provides rapid restoration of tissue function, and is clinically translatable. This is obviously an ambitious goal; however, significant progress have been made in recent years; and further advances in materials design and technology will pave the way for creating significantly custom-made cellular environment for cartilage regeneration.

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**Key words:** Classification of biomaterials, tissue engineering, cartilage repair, tissue regeneration, biomimetic

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**Özet**

Eklem kıkırdak hasarlarının iyileşmesi çok zayıftır ve dejeneratif artrite neden olur. Cerrahi olmayan mevcut tedaviler kıkırdak iyileşmesinde pek sonuç vermediği için, otograf ameliyatı ile vücudun başka bir yerinden alınan kıkırdak ile yenilenmesi kaçınılmaz olmaktadır. Ancak, otograflama işlemi için kullanılabilecek eklem kıkırdağı kaynağının çok sınırlı olması; hem araştırma hem de klinik uygulamalar için kıkırdak yenilenmesini (rejenerasyon) sağlayan malzemelere olan ihtiyacı öne çıkarmıştır. Doku mühendisliğinin ana konusu biyomalzeme iskelelerde hücre büyütme işlemidir. Büyütülen hücreler kıkırdak hasarının olduğu yere, biyolojik yenilenmeyi sağlamak üzere enjekte edilir. Bu makalede önce tıpta kullanılan malzemelerin (biyomalzemeler) sınıflandırılması, biyomalzemelerin tasarımları ve beklenen özellikleri, tedavilerdeki kullanımları hakkında genel bilgiler özetlenmiştir. Takiben; kas-iskelet sistemindeki sorunların yenilenme ile tedavisi yönündeki araştırmalara örnek olarak, biyomalzeme ve doku mühendisliği araştırmalarında en zor alanlardan biri olan kıkırdak doku mühendisliğindeki son gelişmeler, iskele malzemesi olarak geliştirilen biyomalzemelere ağırlık verilerek, tanıtılmaya çalışılmıştır. Kıkırdak yenilenmesinde nihai amaç, biyobenzetim ilkelerini kullanan bir sistem geliştirerek, doğal dokuya benzer özelliklere sahip, doku işlevini hızla yerine getiren ve klinik olarak uygulanabilen kıkırdak dokusu üretilmesidir. Bu amaca tam olarak ulaşmanın ne kadar zor olduğu aşikardır. Buna rağmen, son yıllarda oldukça önemli ilerlemeler kaydedilmiş olup, malzeme tasarım ve teknolojilerindeki yeni ilerlemelerin; kıkırdak yenilenmesinde kullanılmak üzere özel uygulamalara yönelik hücre büyütme koşullarını sağlaması beklenmektedir.

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**Anahtar sözcükler:** Biyomalzemelerin sınıflandırılması, doku mühendisliği, kıkırdak onarımı, doku yenilenmesi, biyobenzetim

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## Contents



## 1. Introduction

Materials scientists and engineers have been able to develop new materials and modify physical, chemical, mechanical, electronic, magnetic and optical properties of new or existing materials to meet the ever increasing demands for more advanced and/or tailor-made materials for specific applications. These developments in materials science and materials technology have been central to many technological advances in all areas of our modern civilization including communication, transportation, energy, construction, aerospace, defense, and health care sectors. Researches on interrelation between structure, processing, properties and service conditions of materials intended to be utilized in biological applications have also been increasing in number and funds to further increase the quality of lives of human beings by providing novel materials or modifying the properties of existing ones with more functionality and biocompatibility. Biomaterials have already being used in a range of established medical applications, including implants to replace diseased joints, surgical-repair materials such as sutures and repair meshes, and tissue such as breast implants. For these established products, continuing R&D will improve key requirements, such as more durable joint implants. Further developments in biomaterials' design and biocompatibility will enable production of novel implant structures. Biomaterials having properties that enhance drug delivery and provide technologies for alternative delivery routes and release mechanisms make a significant contribution in the fast-growing field of drug-delivery systems (DDS). Finely tuned drug delivery is becoming a reality with the support of biomaterials, particularly for the growing range of protein therapeutics emerging from research in genomics and proteomics.

The efforts are not limited to those, and new materials that would help repair or regenerate natural organs have also been under continuous development by research teams consisting of materials scientists, chemists, biotechnologists, engineers and clinicians. These studies have led to the development a multi/interdisciplinary field named as tissue engineering (TE).

For the successful tissue regeneration and repairing, it is indispensable to provide cells with a local environment which enables them to efficiently proliferate and differentiate, resulting in cell-induced tissue regeneration. Biomaterials play an important role in the creation of this regeneration environment in terms of the cells scaffold of artificial extracellular matrix (ECM) and the delivery technology of bio-signaling molecules to enhance the cells potential for tissue regeneration with, in some instances, utilization of stem cell technology. In addition, biomaterials give cells culture conditions suitable for their in vitro proliferation and differentiation to obtain a large number of cells with a high quality for cell transplantation therapy. Cells can be genetically engineered to activate the biological functions by using the non-viral carrier of biomaterials (1).

Articular cartilage, also known as hyaline cartilage, is a tough, extremely smooth, elastic tissue that covers the ends of bones in joints, enabling the bones to move smoothly over one another. Since articular cartilage has no direct blood supply, when it is damaged through injury or a lifetime of use, it does not heal as effectively as other tissues in the body. Instead, the damage tends to spread, allowing the bones eventually to rub directly against each other and resulting in pain and reduced mobility. Significantly damaged cartilage may progress to a more serious condition, such as osteoarthritis (OA). It is estimated that as many as two million Americans damage the cartilage in their knee each year, often through sports, traumatic injuries and work injuries. Additionally, an estimated 27 million Americans suffer from osteoarthritis (OA), also known as "wear-and-tear" arthritis, a chronic degenerative joint disease characterized by the breakdown of the joint's articular cartilage (2).

The knee is the most common joint affected by osteoarthritis, affecting millions of people all over the world. OA of the knee has a significant impact on daily living and it is one of the five leading causes of disability among elderly men and women. The risk for disability from osteoarthritis of the knee is as great as that from cardiovascular disease.

Conventional treatments of articular cartilage (medication, autografting, and total knee replacement) are in temporary in nature, hence put additional burden on health care systems and economies in all countries. The impetus behind the researches on the regenerative solutions to musculoskeletal problems lies in the above factors.

This review aims to provide a brief introduction to the types of materials in medicine (biomaterials), and to design factors and general requirements of biomaterials, and attempts to sum up the recent advances in engineering articular cartilage, one of the most challenging area of study in biomaterials based tissue engineering as an example to the research on regenerative solutions to musculoskeletal problems.

## 2. Materials in Medicine, Their Classification, and Definition of Terms

Biomaterials applications were as far back as ancient Phoenicia where loose teeth were bound together with gold wires for tying artificial ones to neighboring teeth. Bone plates were successfully implemented to stabilize bone fractures and to accelerate their healing in the early 1900's. While by the time of the 1950's to 60's, blood vessel replacement were in clinical trials and artificial heart valves and hip joints were in development (3).

Engineering materials are classified in three main groups: Metals, ceramics and polymers. A fourth group is often added to this classification, composite materials which are made by combining at least two of the three main classes. The properties and characteristics of materials play important roles in almost every modern engineering design, providing problems as well as opportunities for new invention, and setting limits for many technological advances (4). Medical technology is no exception to this statement, and many engineering materials in all four groups have found applications as well in medicine and dentistry. Any material that is used to make reliable, economic and physiologically acceptable devices to substitute a part or a function of the body can be assessed as a "biomaterial".

### 2.1. Biomaterials

In the scope of this review, a biomaterial is any "synthetic material" that is used to replace or restore function to a body tissue and is continuously or intermittently in contact with body fluids or living tissue (5).

This description excludes many materials used for devices such as surgical and dental instruments although they are exposed to body fluids; they are not used to replace part of a living system or to function in intimate contact with living tissue.

Materials used for external prostheses or devices; such as hearing aids and artificial limbs, are also excluded from the above definition of biomaterials as the skin acts as a barrier with the external world and hence, they are not exposed to body fluids although they are in contact with the skin. These materials are referred as "artificial materials".

Materials of biological origin are referred as "biological or natural materials", and wood, skin, artery, collagen and bone are common examples for such materials.

Materials in medicine can then broadly be classified as: i) Biological materials; and ii) Synthetic Biomaterials. Biological materials can be further classified into soft and hard tissue types. In accord to the above definitions, synthetic materials are further classified into: a) Metallic; b) Polymeric; c) Ceramic; and d) Composite biomaterials, Table 1 shows these classifications and some examples for each class (6, 7).

Biomaterials are placed within the interior of the body as implied in its definition of exposure to body

**Table 1.** Classification of materials in medicine and representative examples

| I. BIOLOGICAL MATERIALS (natural) | II. BIOMATERIALS (synthetic) |
|---|---|
| | **Ceramic** |
| | Alumina ($Al_2O_3$), Zirconia ($ZrO_2$), Carbon, |
| **Soft Tissue** | Hydroxylapatite [$Ca_{10}(PO_4)_6(OH)_2$], |
| Tendon, Skin, Cornea, Pericardium | Tricalcium Phosphate [$Ca_3(PO_4)_2$], |
| | Calcium Aluminate [$Ca(Al_2O_4)$], |
| | Bioglass[$Na_2O(CaO)(P_2O_3)(SiO_2)$] |
| | **Metallic** |
| | Stainless Steel, Titanium Alloys (Ti-Al-V), |
| | Cobalt Alloys (Co-Cr-Mo), Platinum, Gold, Silver |
| **Hard Tissue** | **Polymeric** |
| Dentine, Bone, Cuticle | Ultra High Molecular Weight Polyethylene (UHMWPE), Polyurethane (PU), |
| | Polymethylmethacarylate (PMMA), |
| | Polyethyletherketone (PEEK), Silicone, |
| | Polytetrafluoroethylene (PTFE) |
| | **Composite** |
| | Carbon Fiber CF/PEEK, Zirconia/Silica/BIS-GMA, CF/PMMA, CF/UHMWPE |

fluids. Thus, a biomaterial must be biocompatible, nontoxic and noncarcinogenic, as well as they must have adequate physical and mechanical properties to fulfill their anticipated use. Many available engineering materials are not qualified as biomaterials due to the requirement of placement within the body interior.

A number of devices and materials such as tooth fillings, needles, sutures, bone plates, catheters, etc. are used in the treatment of disease or injury (8, 9) and Table 2 provides a brief listing of synthetic materials used for implantation.

Trauma, degeneration and diseases often make surgical repair or replacement necessary. When a person

**Table 2.** Some medical and dental applications of biomaterials

| **Metals and alloys** General Characteristics: Strong, tough, ductile, but prone to corrosion, dense, difficult to make | |
|---|---|
| **Type** | **Principal applications** |
| 316L stainless steel | Fracture fixation, stents, surgical instruments |
| CP-Ti, Ti-Al-V, Ti-Al-Nb, Ti-13Nb-13Zr, Ti-Mo-Zr-Fe | Bone and joint replacement, fracture fixation, dental implants, pacemaker encapsulation |
| Co-Cr-Mo, Cr-Ni-Cr-Mo | Bone and joint replacement, dental implants, dental restorations, heart valves |
| Ni-Ti | Bone plates, stents, orthodontic wires |
| Gold alloys | Dental restorations |
| Silver products | Antibacterial agents |
| Platinum and Pt-Ir | |
| Hg-Ag-Sn amalgam | Electrodes, Dental restorations |
| CP-Ti: commercially pure titanium | |

| **Polymers** General Characteristics: Resilient, easy to fabricate, but not strong, deforms with time, may degrade | |
|---|---|
| **Type** | **Principal applications** |
| Polyethylene | Joint replacement |
| Polypropylene | Sutures |
| PET | Sutures, vascular prosthesis |
| Polyamides | Sutures |
| PTFE | Soft-tissue augmentation, vascular prostheses |
| Polyesters | Vascular prostheses, drug delivery systems |
| Polyurethanes | Blood-contacting devices |
| PVC | Tubing |
| PMMA | Dental restorations, intraocular lenses, joint replacement (bone cements) |
| Silicones | Soft-tissue replacement, ophthalmology |
| Hydrogels | Ophthalmology, drug-delivery systems |
| PET (Dacron): polyethylene terephthalates; PTFE (Teflon): polytetra fluoroethylenes; PVC: polyvinyl chlorides; PMMA: polymethyl methacrylate | |

| **Ceramics and glasses** General Characteristics: very biocompatible, but brittle, not resilient, weak in tension | |
|---|---|
| Type | Principal applications |
| Alumina | Joint replacement, dental implants |
| Zirconia | Joint replacement |
| Calcium phosphates | Bone repair and augmentation, surface coatings on metals |
| Bioactive glasses | Bone replacement |
| Porcelain | Dental restorations |
| Carbons | Heart valves, percutaneous devices, dental implants |

| **Composites** General Characteristics: Strong, tailor- made, but difficult to make | |
|---|---|
| Type | Principal applications |
| BIS-GMA-quartz/silica filler | Dental restorations |
| PMMA-glass fillers | Dental restorations (dental cements) |
| BIS-GMA: bisphenol A-glycidyl | |

has a joint pain the main concern is the relief of pain and return to a healthy and functional life style. This usually requires replacement of skeletal parts that include knees, hips, finger joints, elbows, vertebrae, teeth, and repair of the mandible. The worldwide biomaterials market is valued at close to $\$24x10^9$. Orthopedic and dental applications represent approximately 55% of the total biomaterials market. Orthopedics products worldwide exceeded $13 billion in 2000, an increase of 12 percent over 1999 revenues. Expansion in these areas is expected to continue due to number of factors, including the ageing population, an increasing preference by younger to middle aged candidates to undertake surgery, improvements in the technology and life style, obesity, a better understanding of body functionality, improved aesthetics and need for better function (3).

### 2.2. Classification of Biomaterials Based on Material-Tissue Interactions

It is a fact that no foreign material placed within a living body is completely compatible. The only substances that conform completely are those manufactured by the body itself (autogenous) and any other substance that is recognized as foreign, initiates some type of reaction (host-tissue response). Thus, when a synthetic material is placed within the human body, tissue reacts towards the implant in a variety of ways depending on the material type. The mechanism of tissue interaction, if any, depends on the tissue response to the implant surface. In general, a biomaterial may be described in or classified in three groups based on the tissues responses (10). These are bioinert, bioresorbable, and bioactive, which are well covered in range of review papers (11-13).

#### 2.2.1. Bioinert Biomaterials

The term bioinert refers to any material that once placed in the human body has minimal interaction with its surrounding tissue. Examples of these include stainless steel, titanium, alumina, partially stabilized zirconia (PSZ), and ultra high molecular weight polyethylene (UHMWPE). Generally a fibrous capsule might form around bioinert implants hence its biofunctionality relies on tissue integration through the implant.

#### 2.2.2. Bioactive Biomaterials

Bioactive refers to a material, which upon being placed within the human body interacts with the surrounding bone and in some cases, even soft tissue. This occurs through a time dependent kinetic modification of the surface, triggered by their implantation within the living bone. An ion exchange reaction between the bioactive implant and surrounding body fluids results in the formation of a biologically active carbonate apatite (CHAp) layer on the implant that is chemically and crystallographically equivalent to the mineral phase in bone. Prime examples of these materials are synthetic hydroxyapatite $[Ca_{10}(PO_4)_6(OH)_2]$, glass ceramic A-W and bioglass.

#### 2.2.3. Bioresorbable Biomaterials

Bioresorbable refers to a material that upon placement within the human body starts to dissolve (resorbed) and slowly replaced by advancing tissue (such as bone). Common examples of bioresorbable materials are tricalcium phosphate $[Ca_3(PO_4)_2]$ and polylactic–polyglycolic acid copolymers. Calcium oxide, calcium carbonate and gypsum are other common materials that have been utilized since 1970's.

### 2.3. Design Factors and General Requirements of Biomaterials

Biomaterials are used in the treatment or management of a disease, condition, or injury to improve human health by restoring the function of natural living tissues and organs in the body. Therefore, a sound understanding of relationships among the properties, functions, and structures of biological materials is essential. From this point, biological materials, implant materials, and interaction between these two in the body are the three aspects of the study of biomaterials.

Even in the preliminary stages of this field, surgeons and engineers identified materials and design problems that resulted in premature loss of implant function through mechanical failure, corrosion or inadequate biocompatibility of the component. Key factors in a biomaterial usage are its biocompatibility, biofunctionality, and availability to a lesser extent.

Biomaterials must have special properties that can be tailored to meet the needs of a particular application. For example, a biomaterial must be biocompatible, non-carcinogenic, corrosion-resistant, and has low toxicity and wear (9,14). However, depending on the application, differing requirements may arise. Sometimes these requirements can be completely opposite. In tissue engineering of the bone, for instance, the polymeric scaffold needs to be biodegradable so that as the cells generate their own extracellular matrices, the polymeric biomaterial will be completely replaced over time with the patient's own tissue. In the case of mechanical heart valves, on the other hand, we need materials that are biostable, wear-resistant, and do not degrade with time. Materials such as pyrolytic carbon leaflet and titanium housing are used because they can last at least 20 years or more.

Metals are generally very stiff and have high fracture toughness. In sharp contrast to the metals are the polymers, which have low stiffness and fracture toughness. However the polymers have high elongation to failure. The high stiffness of metals, on the other hand, can be a disadvantage since this can give rise to "stress shielding"

in bone fracture repair. Stress shielding is a phenomenon where bone loss occurs when a stiffer material is placed over the bone. Bone responds to stresses during the healing process. Since the stress is practically shielded from the bone, the density of the bone underneath the stiffer material decreases.

United States Food and Drug Administration (FDA) approval should be obtained for the materials to be used in vivo. A new material is subjected to a series of biocompatibility tests in order to be FDA approved. Biocompatibility requirements in general include acute systemic toxicity, cytotoxicity, hemolysis, intravenous toxicity, mutagenicity, oral toxicity, pyrogenicity and sensitization. There are stringent data and documentation requirements for all tests. Good Manufacturing Practice (GMP) should be adhered to and this requires production should be carried out in completely isolated clean rooms, which is a manufacturing cost increase factor yet an indispensible requirement.

Mechanics and dynamics of tissues and the resultant interactions between them are also an important area, known as biomechanics. Many sophisticated analyses can be made through the use of finite element modeling and analysis (FEM and FEA). These approaches, for example, help to design better prosthesis or customize them for a particular application.

It is imperative that we should know the fundamentals of materials before we can utilize them properly and efficiently. Meanwhile, we also have to know some fundamental properties and functions of tissues and organs. The interactions between tissues and organs with manmade materials have to be more fully elucidated. Fundamentals-based scientific knowledge can be a great help in exploring many avenues of biomaterials research and development.

The study of the relationships between the structure and physical properties of biological materials is as important as that of biomaterials, but traditionally this subject has not been treated fully in biologically oriented disciplines. This is due to the fact that in these disciplines workers are concerned with the biochemical aspects of function rather than the physical properties of materials.

Research on developing new biomaterials is an interdisciplinary effort, often involving collaboration among materials scientists and engineers, biomedical engineers, biotechnologists, pathologists, and clinicians to solve clinical problems. The design or selection of a specific biomaterial depends on the relative importance of the various properties that are required for the intended medical application. Physical properties that are generally considered include hardness, tensile strength, modulus, and elongation; fatigue strength, which is determined by a material's response to cyclic loads or strains; impact properties; resistance to abrasion and wear; long-term dimensional stability, which is described by a material's viscoelastic properties; swelling in aqueous media; and permeability to gases, water, and small biomolecules. In addition, biomaterials are exposed to human tissues and fluids, so that predicting the results of possible interactions between host and material is an important and unique consideration in using synthetic materials in medicine. Two particularly important issues in biocompatibility are thrombosis, which involves blood coagulation and the adhesion of blood platelets to biomaterial surfaces, and the fibrous-tissue encapsulation of biomaterials that are implanted in soft tissues.

Poor selection of materials can lead to clinical problems. One example of this situation was the choice of silicone rubber as a poppet in an early heart valve design. The silicone absorbed lipid from plasma and swelled sufficiently to become trapped between the metal struts of the valve. Another unfortunate choice as a biomaterial was PTFE (Teflon), which is noted for its low coefficient of friction and its chemical inertness but it has relatively poor abrasion resistance. Thus, as an occluder in a heart valve or as an acetabular cup in a hip-joint prosthesis, PTFE may eventually wear to such an extent that the device would fail. In addition, degradable polyesterurethane foam was abandoned as a fixation patch for breast prostheses, because it offered a distinct possibility for the release of carcinogenic by-products as it degraded.

Besides their constituent polymer molecules, synthetic biomaterials may contain several additives, such as unreacted monomers and catalysts, inorganic fillers or organic plasticizers, antioxidants and stabilizers, and processing lubricants or mold-release agents on the material's surface. In addition, several degradation products may result from the processing, sterilization, storage, and ultimately implantation of a device. Many additives are beneficial; for example, the silica filler that is indispensable in silicone rubber for good mechanical performance or the antioxidants and stabilizers that prevent premature oxidative degradation of polyetherurethanes. Other additives, such as pigments, can be eliminated from biomedical products. In order to achieve a "medical-grade" biomaterial, the polymer may need to be solvent-extracted before use, thereby eliminating low-molecular-weight materials. Generally, additives in polymers are regarded with extreme suspicion, because it is often the additives rather than the constituent polymer molecules that are the source of adverse biocompatibility (15).

### 2.4. Nanotechnology Effects on Biomaterials Development

Nanotechnology is a rapidly evolving field that involves material structures on a size scale around 100 nm or less. New areas of biomaterials applications may develop using nanoscale materials or devices. For example, drug delivery methods have made use of a

microsphere encapsulation technique. Nanotechnology may help in the design of drugs with more precise dosage, oriented to specific targets or with timed interactions. Nanotechnology may also help to reduce the size of diagnostic sensors and probes. Transplantation of organs can restore some functions that cannot be carried out by artificial materials, or that are better done by a natural organ. For example, in the case of kidney failure many patients can expect to derive benefit from transplantation because an artificial kidney has many disadvantages, including high cost, immobility of the device, maintenance of the dialyzer, and illness due to imperfect filtration. The functions of the liver cannot be assumed by any artificial device or material. Liver transplants have extended the lives of people with liver failure. Organ transplants are widely performed, but their success has been hindered due to social, ethical, and immunological problems. Since artificial materials are limited in the functions they can perform, and transplants are limited by the availability of organs and problems of immune compatibility, there is current interest in the regeneration or regrowth of diseased or damaged tissue. *Tissue engineering* refers to the growth of a new tissue using living cells guided by the structure of a substrate made of synthetic material. This substrate is called a *scaffold*. The scaffold materials are important since they must be compatible with the cells and guide their growth. Most scaffold materials are biodegradable or resorbable as the cells grow. Most scaffolds are made from natural or synthetic polymers, but for hard tissues like bone and teeth ceramic materials such as calcium phosphate compounds can be utilized. The tissue is grown in vitro and implanted in vivo. There have been some clinical successes in repair of injuries to large areas of skin, or small defects in cartilage. Following section is a discussion on tissue engineering for finding solutions to musculoskeletal health problems, an area of current research activity.

## 3. Biomaterials-based Tissue Engineering and Regenerative Solutions to Musculoskeletal Problems

Loss of human tissues or organs is a devastating problem for the individual patient. Despite technological advances in biomaterials engineering the need for organ and tissue replacement is on rise.

Current technology for organ and tissue replacement has limitations. These include donor scarcity, adverse immunological response from the host tissue, biocompatibility, infection, pathogen transfer, and high cost to patient. Then, there is the perennial deficiency of synthetic material to provide the multifunctional requirement of organ. For example, bone is not just a structural element but also a "factory to produce bone marrow". These limitations prompt scientists worldwide to consider alternative technologies, amongst which tissue engineering has been heralded as the promising answer. This is considered as a paradigm shift from "finding replacements/substitutes to human tissue" to "trying to have the lost human tissue re-grow".

The term "tissue engineering" has now come to encompass a wide range of strategies employing cells, synthetic and processed natural materials, tissues, cytokines and genes for the regeneration of tissue *in vivo* or the production of tissue *in vitro*. Cell therapies and tissue transplant procedures are thus now often considered under the rubric of tissue engineering (16).

The aim of tissue engineering is to restore tissue and organ functions with minimal host rejection. This arose from the need to develop an alternative method of treating patients suffering from tissue loss or organ failure. TE has been heralded as the new wave to revolutionize the healthcare-biotechnology industry. It is a multidisciplinary field and involves the integration of engineering methods and principles, basic life sciences, and molecular cell biology towards the fundamental understanding of structure-function relationships in normal and pathological mammalian tissue and the development of biological substitutes to restore, maintain, or improve tissue function

The success of tissue engineering lies in five key technologies. They are namely: a) Biomaterials; b) Cells; c) Scaffolds; d) Bioreactors; and e) Medical imaging technology. It may seem simple to produce a one-off, tissue-engineered product in the laboratory, but it is a completely different matter to produce hundreds of products of consistent quality for clinical use.

Fundamentally, TE involves a scaffold which acts as a temporary extracellular matrix for the cells to adhere to, differentiate and grow. Breakthrough has been made in the development of a platform technology which integrates medical imaging, computational biomechanics, biomaterials, and advanced manufacturing to produce three-dimensional porous load bearing scaffolds for tissue engineering of bone (17). The technology makes use of polycaprolactone (PCL) bioresorbable polymer and Fused Deposition Modeling's (FDM) rapid prototyping advanced manufacturing fabrication process to produce the scaffolds without a mold (18). Controlled three dimensional architecture with interconnected pores enables good cells entrapment and facilitates easy flow path for nutrients and waste removal, and demonstrates long-term cell viability. Patient-specific scaffolds can now be made using this technology. This biomaterial processing technology has paved the way for patient-specific tissue engineering concepts not dreamed of a few years ago.

Scaffolds are temporary materials structured in such a way as to guide cell growth in vitro or in vivo. Cell growth in a scaffold (matrix) can be facilitated by other biological entities such as growth factors. Scaffold materials, cells, and soluble cell regulators have to work together to achieve optimal regeneration of tissues and organs. So called "TE triad" is shown in Figure 1.

In this triad, the cell is the main character to generate tissue. Native, stem or allogenic cells produce ECM to constitute tissue. Porous scaffolds of adequate structural strength made from absorbable biomaterials provide the habitation for the cells. Cell behavior is controlled by the environmental factors including chemical, physical and biological variables and substances in the culture system (19).

Materials for the scaffolds used in TE includes a variety of absorbable or nonresorbable synthetic and natural materials that are used together with a variety of cells and regulators for tissue regeneration.

Another approach to the tissue or organ replacement is reproduction of the whole organ or body by cloning from somatic cells rather than reproductive cells. This is how "Dolly" the sheep was reproduced. The spare body part concept may not be acceptable in humans due to ethical as well as technical problems. Some application examples (20) of TE are given in Table 3.

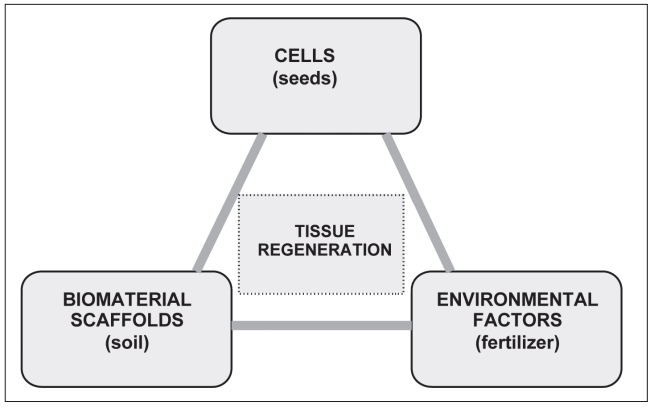


**Figure 1. The tissue engineering triad (adapted from 9)**

**Table 3.** Some application examples of Tissue Engineering

| Application | Example |
|---|---|
| Cell production in vitro | Bone marrow cell production |
| Extra corporeal devices | Artificial liver |
| Tissue growth and repair in situ | Nerve regeneration |
| | Artificial skin |
| | Blood vessel |
| | Bone and cartilage |
| Implantable devices | Endotherialized vascular grafts |
| | Bone and cartilage implants |
| | Artificial pancreatic islets |
| | Skin generation template |

### 3.1. Tissue Engineering for Articular Cartilage

One major research area of TE is the cartilage tissue of joints. The degeneration of articular cartilage and associated arthritis (osteoarthritis and rheumatoid arthritis) is among the most prevalent chronic condition in all over the world. In osteoarthritis, the cartilage covering the joint gradually wears away, exposing the bone which in turn makes joint motion difficult and painful. The condition is overwhelmed by two major drawbacks: First, the regenerative capacity of cartilage tissue is limited due to the sparse population of chondrocytes, reduced presence of progenitors and the avascular nature of the tissue. Second, the repaired tissue that is formed is a combination of hyaline and fibro-cartilage that has poor mechanical properties when compared to native articular cartilage and also tend to degrade over time. Therefore, one early clinical finding in 1743 by Hunter on articular cartilage that "once destroyed, is not repaired" has not been much changed (21).

Conventional treatment methods of osteoarthritis or damaged cartilage tissue includes: drug therapy that often involves anti-inflammatory drugs and analgesics (first line of treatment); surgical intervention/manipulation wherein autologous chondrocyte implantation (ACI), subchondral drilling, mosiacplasty, and allografts are introduced (second line of treatment); and finally total knee replacement which involves the replacement of the arthritic knee with an artificial knee (third line of treatment). The first line of treatment provides only symptomatic relief. The second line of treatment often leads to the formation of fibrocartilage that is mechanically inferior to articular cartilage. The third line of treatment is a potential solution, however, it involves a major surgery and the implants are very costly. Besides, the life of a knee replacement implant is in the range of 10-15 years.

TE provides an opportunity to overcome the limitations associated with conventional treatment methods of cartilage tissue loss. As stated, TE triad includes a scaffolding system, tissue specific or progenitor cells and growth factors (cell signaling molecules). The scaffolding system is central to TE strategy as they provide cells with a surface for adherence and 3D growth. Scaffolds can also be used as a reservoir for growth factors that can be delivered locally for a specific duration at a suitable rate. Cartilage tissue is made up of a small population of cartilage cells (chondrocytes) and largely extra-cellular matrix (ECM) that is in turn mainly made up of type II collagen and glucoaminoglycans (GAGs). These ECM components are fibrous in nature and have diameters in nanometer scales. From biomimetic approach, studies are concentrated to develop nanofibrous 3D scaffolds that mimic the type II collagen and GAG fibrils (22). The nanofibrous scaffolds can be fabricated using the electrospinning technique that involves the application of

a high voltage field (up to 10 kV/cm) to a polymer at the tip of a needle by virtue of its viscosity. The polymer solution is then provided with a voltage potential that in turn provides charge to the polymer solution. As the potential gradually increased the charge density on the polymer solution increases and eventually leads to columbic repulsion. When the repulsive forces exceed the viscous forces of the polymer, a jet ensues from the tip of the needle that initially has a straight path and then undergoes instabilities to traverse a spiral path with increasing diameter. This trajectory of the jet allows for continuous thinning of the polymer jet as well as evaporation of the solvent from the jet, eventually leading to the formation of charged dry nanofibers that are collected on a grounded metallic collector. The fibers obtained using the electrospinning technique can be altered both in terms of morphology and diameter via modification in the fabrication parameters. The morphology can vary from elliptical bead containing fibers to smooth fibers and the diameters can range from 10's to 1000's nanometers. In one approach it is proposed to use the nanofibrous scaffolds as growth factor delivery system, wherein the growth factors will be linked to the scaffolding system covalently using a linker molecule. This system when implanted into an arthritic knee will be exposed to proteases (enzymes that selectively cleave specific bonds) that will cleave the covalent bond that connects the growth factor to the nanofibrous scaffold, thereby leading to the release of the growth factor. It is expected that the released growth factor will then provide the necessary signaling to enable enhanced cell proliferation and function. Some nanofibers systems can also be applied to other applications such as filter media, sensors, electrically conducting nanofibers, optical applications, material reinforcement, protective clothing and cosmetics (23).

### 3.1.1. Scaffolds

Biomaterials are used for promoting cartilage repair by providing scaffolds for cell attachment, growth and differentiation and could act as vehicles for protein and gene delivery to regenerate functional tissue. For cartilage, biomaterials should have several properties to support viable repair.

Numerous scaffolding materials have been used for cell delivery in cartilage regeneration. The primary focus has been on both natural and synthetic polymers, in a variety of forms (24). These include hydrogels, sponges, and woven or non-woven fibrous meshes as shown in Figure 2. Scaffold architecture plays a major role in dictating cellular behavior; therefore, a scaffold architecture that mimics the natural environment may facilitate the growth of seeded chondrocytes. A layered agarose scaffold with such depth-dependent nonhomogeneity has been designed for good *in vitro* regeneration of cartilage from chondrocytes (25).

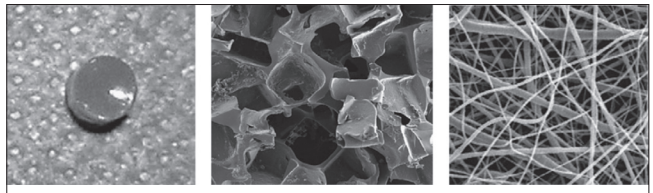


**Figure 2. Examples of different scaffold architectures used in the engineering of cartilage tissues (24)**

Scaffolds provide a 3D environment that is desirable for the production of cartilaginous tissue. Ideally the scaffold should: 1) have directed and controlled degradation, 2) promote cell viability, differentiation, and ECM production, 3) allow for the diffusion of nutrients and waste products, 4) adhere and integrate with the surrounding native cartilage, 5) span and assume the size of the defect, and 6) provide mechanical integrity depending on the defect location (24). Scaffold degradation can occur hydrolytically or enzymatically, and by controlling degradation temporally and spatially, scaffolds can enhance and direct new tissue growth. For example, scaffolds with degradable and non-degradable units show improved ECM distribution compared to completely non-degradable scaffolds (26) However, a balance must be found since slow degradation may impede new cartilaginous ECM production, while fast degradation may compromise structural support and shape retention. For instance, Solchaga et al. (27) showed that scaffolds with slower degradation rates yielded cartilage of greater thickness in an osteochondral defect model, but cracks and fissures were evident on the cartilage surface.

Natural materials that have been explored to produce scaffolds for cartilage engineering to date include: collagen, fibrin, hyaluronic acid (HA), alginate, agarose, gelatin, chitosan, chondroitin sulfate, silk, and cellulose (28-35). Advantages of natural materials are that many of them are natural bodily constituents that provide a natural adhesive surface for cells and carry the required information for their activity. Furthermore, the degradation products are physiological ones and therefore non-toxic. The major disadvantages of natural polymers include sourcing, processing and possible disease transmission. In addition, natural polymers may be inferior mechanically and subject to variable enzymatic host degradation (24, 36).

Synthetic polymers are more controllable and predictable, where chemical and physical properties of a polymer can be modified to alter mechanical and degradation characteristics (design flexibility), and eliminates disease transmission. Synthetic polymers currently explored for

cartilage repair include: poly$\alpha$-hydroxy esters such as polylactic acid (PLA) and polyglycolic acid (37, 38). These polymers have been approved for clinical use in the USA and are manufactured for routine hospital or surgical use. They are readily made into scaffolds for tissue engineering, in the form of foam or woven or nonwoven fiber mesh. Products of these polymers have much better mechanical strength than those of natural substances, which makes it easier for them to be fixed to the recipient site, and makes them more resistant to the friction of joint motion (39). Their copolymers allow adjustment of the degradation rate of the scaffold. This is important because the residence time of the implanted polymer must be sufficient to serve its scaffold purpose, but not so long as to impede tissue regeneration. Other polymers of interest include poly(ethylene glycol)-terephthalate, poly (butylene terephthalate), poly (ethylene glycol) fumarate, poly (N-isopropylacrylamide), polyurethanes and carbon fiber scaffolds (40-43). Disadvantages of synthetic polymers are those that, unless specifically incorporated, they do not benefit from direct cell-scaffold interactions, which can play a role in adhesion, cell signaling, directed degradation, and matrix remodeling. In addition, degradation byproducts may be toxic or elicit an inflammatory response. Excellent reviews outlining the advantages and disadvantages of different scaffold structures and scaffold materials are available elsewhere (19, 24, 44), to which interested readers are directed.

#### 3.1.2. Cell Source

The optimal cell source for cartilage tissue engineering is still being identified. Chondrocytes, fibroblasts, stem cells, and genetically modified cells have all been explored for their potential as a viable cell source for cartilage repair (45, 46). Chondrocytes are the most obvious choice since they are found in native cartilage and have been extensively studied to assess their role in producing, maintaining, and remodeling the cartilage ECM. Fibroblasts are easily obtained in high numbers and can be directed toward a chondrogenic phenotype (47, 48). More recent studies have focused on stem cells, which have multi-lineage potential and can be isolated from a plethora of tissues. These progenitor cells can be expanded through several passages without loss of differentiation potential. Additionally, all of these cells can be modified genetically to induce or enhance chondrogenesis. The goal is to find an ideal cell source that can be easily isolated, is capable of expansion, and can be cultured to express and synthesize cartilage-specific molecules (e.g., type II collagen and aggrecan).

#### 3.1.3. Signaling Factors

As the third component of the tissue engineering triad, stimulating factors have been employed to induce, accelerate, and/or enhance cartilage formation. For instance, growth factors and other additives may be added to culture media in vitro or incorporated into scaffolds for in vivo delivery to control cellular differentiation and tissue formation. Regardless of the nature of the cells, standard culture conditions require the presence of serum, basically of bovine origin. The risk of undesired pathogen transmission has been debated when the cells are implanted to humans. Autologous serum-supplemented culture medium has become the state of the art for ACI, but serum-free culture is more attractive (49). The avascular condition of natural cartilage does not suggest that serum is needed to support the chondrocytes. One study has even indicated that serum hinders the chondrogenic ability of chondrocytes (50). Serum-free culture is worthy of further development to develop regenerated cartilage for clinical application.

In addition, gene therapy has emerged as another method of local delivery, where cells can be engineered to over-express bioactive molecules. An additional approach is the introduction of mechanical signals through loading regimes such as hydrostatic or dynamic compression or through the use of bioreactors. Since many types of cartilage depend on mechanical forces to maintain healthy function, this approach has been used to alter cellular differentiation and tissue production.

## 4. Conclusions

The impact of biomaterials on further enhancement of human health seems to be substantial. The success in regenerative medicine appears to lie in the developments of biomaterials based tissue engineering. Cell source, scaffolds, and signaling factors make up the tissue engineering triad. One of the biggest challenges for cartilage tissue engineering is cell source. Current studies on alternatives to chondrocytes are increasing, and the potential and limitations of fibroblasts and stem cells are being investigated. Novel biomaterials are being continuously developed and are leading to distinctive interactions with cells through controlled biomaterial chemistry, structure, and the addition of biological molecules. However, sequences and concentration of growth factors which are needed to optimize cartilage regeneration are not well developed. The incorporation of stimulatory factors such as bioactive molecules, gene therapy, mechanical loading, and bioreactors are being studied to enhanced cartilage production.

Much closer matching of synthetic scaffolds to the natural extracellular matrices can be achieved by development novel biomaterials that meet specific mechanical property requirements and cell-specific interactions via high-throughput, analytical technologies combined with recent advances in genetic engineering.

A successful tissue engineered cartilage product should make it through the regulatory processes. Although there are a number of patented cartilage products, and some companies have already applied for FDA approval; most of the studies on biomaterials were performed using mostly young adult or even fetal animal cells, and not with cells from elderly osteoarthritis patients. All of the approaches without consideration of clinical translation and feasibility needs would be, to some extent, futile activity. In due course, extensive research will be needed to determine whether the results can be extended to the human situation. Therefore, professionals working in this multidisciplinary area have assumed huge responsibility. Expectedly within five years or so, the results and findings of research teams, consisting of an appropriate combination of engineers, clinicians and basic science researchers, will lead to the development of novel biomaterials and devices that will help improve the quality of human lives, if not yet for the creation of "The Six Million Dollar Man".

**Conflict of Interest**

No conflict of interest is declared by the author.

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