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Current Status of Biomaterial Research Focused on Regenerative Medicine

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1 Introduction

Rapid advances in medical technology have made it possible to save the lives of patients who have lost one or more vital organs due to severe disease, such as cancer, or from serious traffic accidents. Many of these patients can even be rehabilitated back into society. Future medical technology strives to restore lost tissue to its original state. Inspired by the famous experiment by Vacanti et al. in the United States, in which auricular cartilage was regenerated using cell-incorporated biomaterials[1], the medical community is experiencing a worldwide boom in "regenerative medicine" research.

Regenerative medicine is a generic term for a variety of therapeutic techniques to restore lost or damaged organs and tissues due to accidents or diseases to their original state, taking advantage of the regenerative capacity inherent in biological entities. Biomaterial research has two lines of investigation: one is to aim at cell/tissue regeneration by means of tissue replenishment, such as bone marrow transplant, and the other is to regenerate tissues using "tissue engineering" techniques. Regenerative medicine is advancing internationally along these two lines[2]. In this context, tissue engineering refers to techniques to restore tissues using cells, scaffolds required to settle in the body, and cell growth factors. Some tissue engineering products have already been brought into practical use in the fields of skin, cartilage and bone regeneration, and their worldwide market size is estimated to be around 17.1 billion yen. If we include regeneration and transplantation of bone, cardiovascular system, teeth and organs, the total worldwide market size is estimated to reach 250 billion yen by 2015[3]. In a separate approach to treat diseases and physical damage with minimal invasion, research on drug delivery system (DDS) and minimum invasive surgery have also shown remarkable progress. These next-generation medical technologies will contribute immensely to improve the quality of life (QOL) of patients, especially those who are elderly. These technologies also contribute to reduce medical costs associated with prolonged admission and visits to hospitals and welfare costs associated with nursing care.

Research in tissue engineering has a worldwide demand, as does Japan, which has a rapidly aging population. In Japan, deployment of medical and welfare measures are highly sought after. Almost every country is engaged in fierce competition for better regenerative medicine. However, the fruits of this research are limited to such areas as skin (basically a two-dimensional tissue) and cartilage (cells occurring in a hypoxic and low-nutrient environment). There still remain many challenges to be solved, such as a significant difference in three-dimensional structure between regenerated and natural tissues/organs.

This article provides an overview of the current status of biomaterial research from the nano level and discusses challenges and solutions for industrialization of biomaterials in Japan.

2 What are Biomaterials?

Biomaterial is a generic term for a variety of materials used for manufacturing artificial organs that have direct contact with cells and tissues for a relatively long period of time, or those used in regenerative medical techniques, which will be discussed in later sections of this paper. These materials include those applying surfaces of the body or connecting the body via tubes, such as contact lenses and artificial dialysis membranes, as well as implantable devices, such as artificial hearts and artificial joints. Figure 1 shows the types of artificial tissues and organs currently used in the clinical setting[4].

An essential requirement for materials that remain in contact with living tissues for a medium to long period of time is that they are not harmful to tissues and biological activities, which is a challenging objective. The toxicity of substances implanted in the body must be considered differently from those taken orally. For example, cooked rice taken by mouth will become nutrients for the human body, but implanting the same cooked rice inside the body (for example, under the skin) will give rise to inflammation. Thus bioaffinity, or biocompatibility of the material, is the essential element for the development of biomaterials.

It is thus particularly important to "use materials with molecular structures that are similar to those found in the body". However, making a clear demarcation is difficult regarding to what extent the pursuit of similarity is effective and beyond which it is dangerous. In organic chemistry, clever combination of partial spatial configuration and functional groups can induce reaction with biomolecules, having inspired a variety of efforts to develop new drugs for alleviating disease symptoms. These drugs, however, may have serious side effects.

Considerable effort has been made to compensate or regenerate lost functionality of living tissues by producing materials and developing novel devices thereof using a selected range of inorganic, organic, and metal materials, giving rise typically in the 1990s to the evolution of the concept of regeneration medicine and tissue engineering. More recently, there is also an effort afoot to produce artificial extracellular substrate that is actually required by cells and

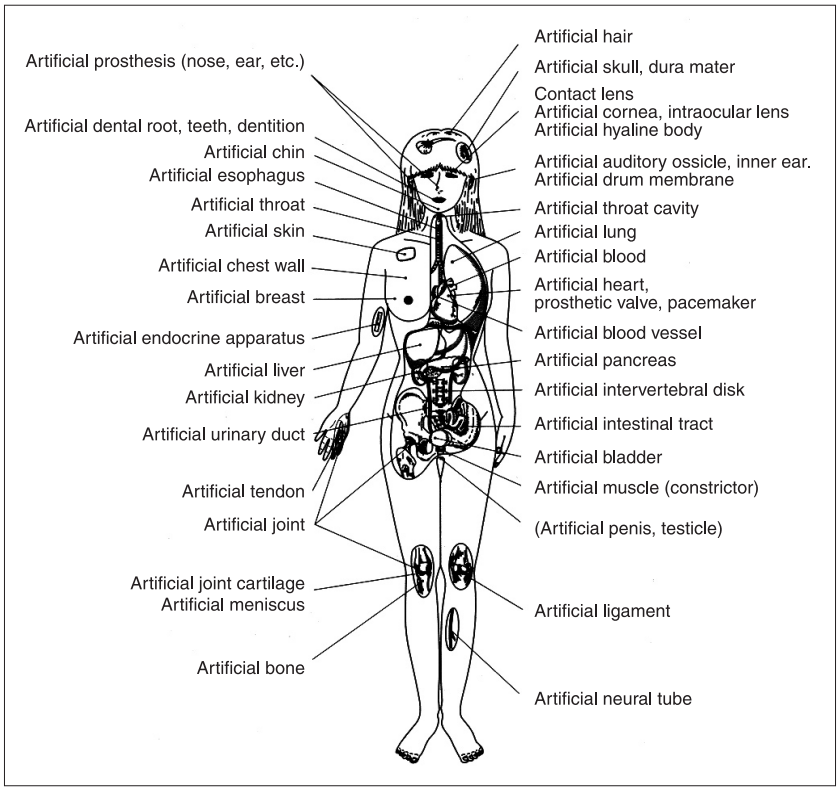

Figure 1 : Prosthetic devices

Quoted from Reference[4], p.15

tissues utilizing nanoscale control in response to needs in the clinical setting. Along this trend, "soft nanotechnology" that does not require extreme conditions (high temperature/pressure, vacuum, etc.) has come into practical use in such areas as artificial bones[5] and drug delivery systems[6]. Application of biodegradable metal materials has also entered into consideration in these areas.

3 Current status of regenerative medicine of body part/organ and biomaterial research

Table 1 summarizes, from the perspective of relatively advanced regenerative medicine research, the challenge level of regeneration and biomaterials used for body parts and organs.

Almost all living tissues are made of organic materials such as proteins, lipids and polysaccharides. Because the method of chemically modifying organic polymers (for example, by introducing functional groups) has been established in many cases, molecular structure design of organic materials is more feasible for organic biomaterials than for other materials, generating a proliferation of organic material research. In the area of inorganic biomaterials, Japan has attained one of the most advanced levels of research in the world. For example, artificial bone using hydroxy apatites*1 was developed in Japan, and artificial body fluids*2 was also first postulated in Japan. In the development of organic-inorganic compound

Table 1 : Challenge level of regeneration of and biomaterials used for tissues/organs

Tissue / Organ	Challenge level of regeneration	Organic material	Inorganic material	Organic-inorganic polymer
Skin	Basically two-dimensional tissue. Culture is relatively straightforward.	Collagen, synthetic biodegradable polymers (polylactic acid, etc.)	—	—
Cartilage	Extracellular matrices have 3-dimensional structures, but there are no blood vessels in cartilage tissues. Cartilage cells are tolerant to low-oxygen, low-nutrient environment, making it relatively easy to perform 3-dementional culture.	Polysaccharides (collagen, chondroitin sulfate), synthetic biodegradable polymers (polylactic acid, etc.)	—	Collagen / polysaccharide, collagen / polysaccharide / hydroxyl apatite
Bone	Extracellular matrices have 3-dimensional structures containing blood vessels. Difficulty in sustaining cellular activity and function in the central porous core.	Collagen, synthetic biodegradable polymers (polylactic acid, etc.)	Calcium phosphate (hydroxyl apatite, β-tricalcium phosphate, α-tricalcium phosphate)	Biodegradable polymers/calcium phosphate, collagen/calcium phosphate
Pancreas	Almost no extracellular matrices exist. The organ has two parts: the exocrine system secreting proteases for digestion and the endocrine system (pancreatic islets) secreting insulin and other hormones. Current research focuses on regeneration of pancreatic islets that produce insulin used for diabetic treatment.	Hydrophilic polymers (polyethylene glycol, etc.) and hydrophobic polymers (coating) for culture plates. Agarose as immunoisolation membrane.	Silica gel hollow beads used as immunoisolation membrane	Agarose / polystylene sulfonate as immunoisolation membrane
Liver	Almost no extracellular matrices exist. Regeneration is difficult because of the extensive vascular networks and large blood flow..	Hydrophilic polymer (polyethylene glycol, etc.) and hydrophobic polymers (coating) for culture plates. Temperature-responsive culture plate for regeneration of 2-dimensional liver cell sheet.	Apatite porous media used for liver cell culture	—
Capillary blood vessel	Regeneration of capillaries is difficult to regenerate because of the small tubular structure consisting of 3 different layers, but capillaries are necessary for survival of regenerated organs. Vascular endothelial cells are the most common target of tissue engineering.	Patterned culture plates capable of regulating cell adhesion. Hydrogel-cell compositions. Synthetic biodegradable polymer nanofibers used as scaffolds for cell culture.	—	—

materials, Japan also occupies a top level in the world.

The following summarizes the current status of research and challenges in roughly the order of attainability.

3-1 Skin

The skin system is comprised of two components: epidermis and dermis, as shown in the accompanying Figure 2[7]. Epidermis, or scarfskin, has a thickness of 0.1-0.2 mm (palms and soles have thicker scarfskin, from 0.8 to 1.5 mm), in which cells originated by cell division in the innermost part move toward the surface, gradually cornifying, and finally fall off from the skin surface. Dermis or inner skin, in contrast, consists of meticulously woven collagen fibers and elastic fibers protecting blood and lymph vessels as well as providing a tough and elastic texture.

Skin can lose its elements due to burn injury and decubitus (bedsores). Skin loss is classified into epidermis loss and full-thickness skin loss, depending on the depth of the layers lost. The former can be effectively cured by grafting cultured skin containing only epidermis cells. Skin generally has the resilient capacity of regeneration and, because of the thin tissue layer, skin rarely has difficulties in culturing such as necrosis of the core due to insufficient exchange of nutrients and gases. Cultured skin has practical worldwide applications, including in Japan. Although artificial skin has not been approved in Japan, we have many cases where patients' own skin is cultured and grafted onto themselves. In emergency cases such as extensive burn, a common practice is to temporarily graft cultured skin from another person and later replace it with the patient's own cultured skin tissues.

For decubitus treatment, a porus collagen sponge layer coated with polymers such as silicon on one side to reinforce the membrane is used to regenerate an artificial epidermis, in which cells and tissues attach on the surface and migrate into internal pores of the collagen sponge layer to produce an epidermis-like layer. For enhancing the efficacy of this treatment, some specialists immerse the collagen layer in bone marrow fluid taken from the patient to induce active tissue regeneration. These methods using collagen sponges can be useful in emergency cases, but subsequent partial skin grafting from other parts of the body or cultured skin grafting is required after the artificial dermis layer has grown.

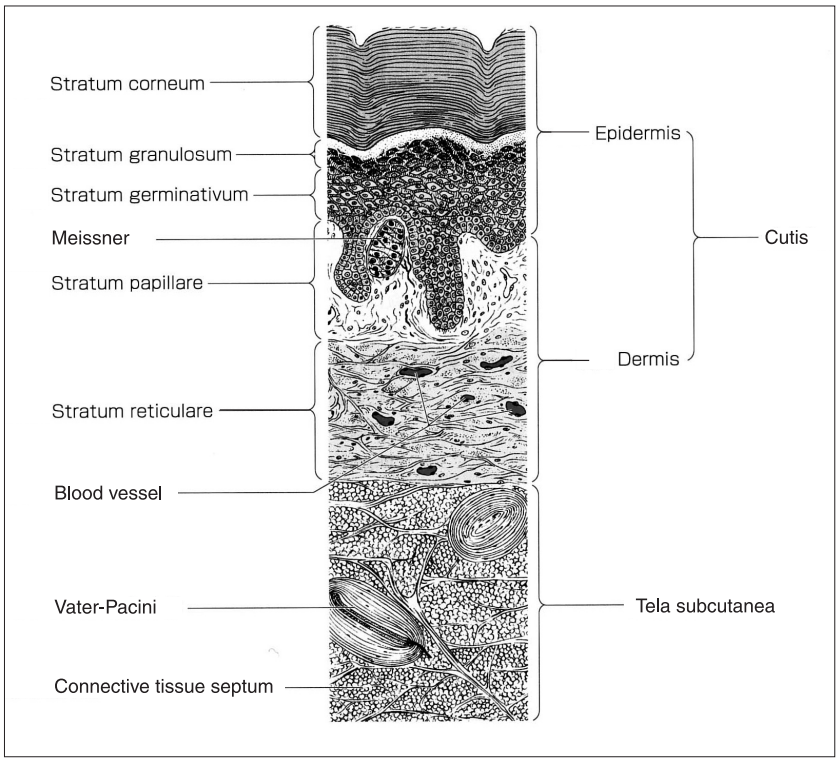

Figure 2 : Cross-sectional view of human skin

Quoted from Reference[7], p.254

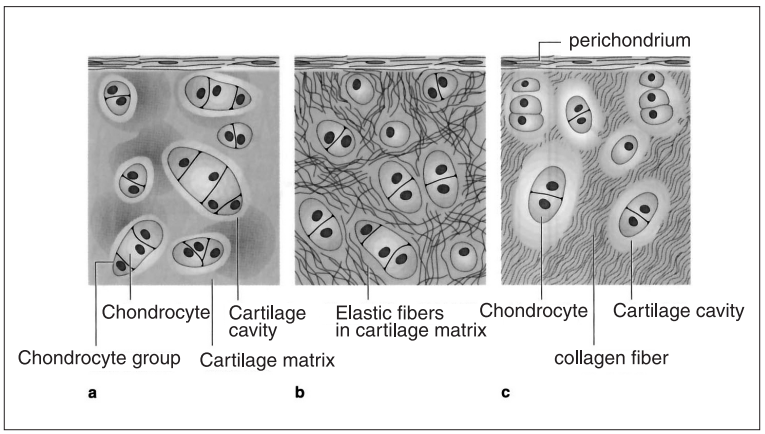

Figure 3 : Schematic view of cartilage tissue

a: hyaline cartilage b: elastic cartilage c: fibrocartilage

Quoted from Reference[7], p.20

Although skin treatment is a relatively advanced area among regenerative medical techniques, it still has many challenges, such as a prolonged treatment period required due to repeated operations.

To shorten the period required for a complete cure, new types of cultured dermis and cultured skin consisting of dermis and epidermis have been developed using collagen as a scaffold material*3. These cultured skin substitutes have shown good results experimentally, and considerable effort is being invested towards commercialization. In Japan, Japan Tissue Engineering Co., Ltd. (J-TEC) and BCS, Inc. are aiming at bringing the technique into clinical application, and the regenerated skin from J-TEC is expected to obtain official approval by the end of 2007. Skin regeneration is in a relatively advanced stage both in technique and materials, but currently available artificial skins does not include skin appendages, such as sweat glands, sebaceous glands and hair follicles. Research and development for regenerating skin containing these appendages and developing suitable scaffold materials is needed. The aesthetic aspect should also be an important consideration for new generation regenerative skins.

The number of patients requiring skin transplantation because of scars or ulcerations in Japan is estimated to be around 35,000, and the forecast is that the number will remain at the same level until 2020. Another forecast indicates that about 30% of these patients will use cultured skin in 2020, and the market size of cases is expected to grow to the level of 5.4 billion yen. Extending cultured skin applications to patients with burn and traumatic skin damage will result in a market size several times larger than this, and further extending applications to severe burns cases will create an estimated 28.5 billion yen market in 2020[8].

3-2 Cartilage

Cartilage consists of cells and substrate, with the cells distributed among the substrate without touching other cells (Figure 3). The cartilage of human adults contains 80% of water and does not have vascular tissues. The cells take in nutrients and oxygen from synovial fluid through the perichondrium, and dispose of wastes and carbon dioxide in reverse direction. Because the amount of nutrients transported using this pathway is much smaller than through blood vessels, chondrocytes generally have greater tolerance against nutrient/oxygen-depleted environment. Organization of cartilage is different for each part of the body: hyaline cartilage (Figure 3-a) is found in joints, fiber-rich elastic cartilage (Figure 3-b) is found in the earlobe, and fibrocartilage (Figure 3-c), which contains type-I collagen and shows strong tolerance against pressure, is found in tendons in meniscuses and interspinal disks and ligament tissues.

Hyaline cartilage tissues mainly consist of polysaccharides such as hyaluronic acid, chondroitin sulfate and keratan sulfate, and collagen. Polysaccharides account for 10% of total dry weight, and collagen 60%. Collagen that holds the shape of hyaline cartilage is type-II, whose ability to form fibers is lower than type-I found in

skin, bone, and fibrocartilage.

Fibrocartilage, if damaged, can be gradually restored, except for the fibrocartilage in blood-depleted area such as meniscuses and interspinal disks. Other cartilages do not have the capacity of spontaneous recovery; surgical procedures must be taken to restore this tissue. Damaged joint cartilages especially need early restoration procedures because they are necessary to absorb shocks and allow the smooth movement of joints, which directly affect the daily activities of the patient*[4].

One of the traditional methods for restoration is to drill a hole through the subchondral bone underlying the damaged joint cartilage into the bone marrow, and introduce precursor cells, nutrients and growth factors to regenerate fibrocartilage. Although this method can restore only the fibrocartilage, it has been widely used because it can provide short-term recovery. However, since the long-term prevalence of arthrosis deformans is high in patients receiving such treatment. the mainstream has shifted to mosaicplasty, a technique of creating an osteochondral autograft by harvesting small cylindrical osteochondral plugs from areas of normal cartilage on less weight-bearing surfaces and inserting them into the defective section of cartilage. This method has the advantage of enabling restoration of hyaline cartilage, but the portion of cartilage from which the autografts are obtained will not restore its original shape and the amount of grafts available is limited.

In order to overcome these problems, a method to restore cartilage using cultured cells, has been commercialized for the first time in the field of orthopedics. A small amount of tissue is obtained from the patient's cartilage in a less weight-bearing region, and the chondrocytes isolated from the tissue are cultured to increase cell numbers, and the suspended chondrocytes are injected into the cartilage defects covered by the patient's own periostea. Remaining problems with this method can be the lowering of activity in cultured cells as chondrocytes and the fact that this method also produces, although small, a defect in the patient's cartilage. One method that has the potential for resolving these problems is to use the patient's own bone marrow mesenchymal cells and make them differentiate to chondrocytes after obtaining a sufficient number of mesenchymal cells through cultivation.

Chondrocytes, being by their nature tolerant to oxygen-, nutrient-deficient environment, are capable of growing well in three-dimensional scaffold materials and producing extracellular substrates. Difficulties in joint cartilage regeneration are focused on the adhesion of cartilage tissues to subchondral bones, and do not include such problems as blood vessel introduction and generation of co-operative environment for different types of cell species (mutual interaction and spatial configuration) as in visceral organs. This makes in vitro cartilage tissue regeneration relatively easy. This is well evidenced in that the experiment by Vacanti et al. that produced a "mouse with a human earlobe on its back" was a straightforward success. Selection of cartilage for the tissue regeneration target was the key to the success of this experiment, and this success opened the door to development of tissue engineering.

In cartilage tissue engineering, chondrocytes or bone marrow mesenchymal cells are multiplied in the scaffold materials suited for this purpose to regenerate, by adding growth factors and mechanical stimuli, cartilage tissue containing appropriate cartilage substrate. Joint cartilage is a supporting organ that always receives mechanical stimuli to support the body. Attempts are also being made to regenerate bone tissue beneath the cartilage tissue to ensure tight adhesion to the subchondral bone.

In most cases, synthetic biodegradable polymers (e.g., polylactic acid and polylactic-glycolic acid copolymer) and collagen sponges have been utilized for scaffold materials; more contributions from biomaterials to cartilage tissue engineering are desired. Gels with similar chemical composition to cartilage substrate (for example, mixtures of type II collagen and hyaluronic acid)[9] have been developed for use as scaffolds for cartilage tissue engineering. However, to cope with a wider range of cartilage defects, the mechanical environment similar to actual cartilage is also important as well as the chemical environment. Composition and

structural characteristics of the scaffold material affects the responses of scaffold and artificial extracellular substrates against mechanical stress, and may result in transmission of inappropriate mechanical stimuli to the cells. Further study of appropriate scaffold materials is needed.

Regeneration of elastic cartilage, such as earlobes, garners attention from its aesthetic aspects, but we have had no elastic cartilage substitutes to date. The most difficult challenge in development of cartilage substitutes could be interspinal disk regeneration. Among cartilage tissues, the interspinal disk has the most complex structure consisting of a central pulpy nucleus and surrounding rings of fibrous cartilage (fibrous rings), with a set of hyaline cartilages sandwiching them from above and below providing connection with bones. Achieving regeneration and functioning of such a complex structure in a relatively short period of time will require the development of suitable scaffold materials and tissues that have been sufficiently matured in an in vitro environment. Joint cartilage has a four-layer structure with each layer having a different cell distribution and microstructure: surface layer, intermediate layer, deep layer (zona radiata), and calcified layer (a layer connecting the cartilage and the underlying subchondral bone). Observation at the nanoscale level shows that the layers have a network of type-II collagen for maintaining its structural integrity and nanoscopic structure for incorporating polysaccharides that help retain water. Development of materials with structural characteristics similar to natural joint cartilages will be required to ensure the development of cartilage function immediately after graft implantation as well as the maintenance of functions of cultured chondrocytes.

We have 500 to 600 thousand patients with degenerative arthritis in Japan and approximately 10% of them are considered treatable by implanting relatively small regenerated cartilage grafts that current technology can provide. This market size is estimated to be approximately 60 billion yen. There is room for extending patient coverage by improving current technology, but the room for the increase of curable patients is estimated to be 20% at most (approximately 100 billion yen market)[8]. If bone regeneration technology is developed for practical use, combination of bone and cartilage therapy embraces nearly all patients, including those who suffer from rheumatoid arthritis (approximately 280 thousand patients), and the market size is likely to reach 1,000 billion yen.

3-3 Bone

Bones carry important mechanical functions of supporting the body and protecting vital organs, such as the brain, heart, lungs and central nervous system. Another important function is to provide storage of calcium and phosphorus. Homeostasis of calcium depends heavily on the bones. Extracellular matrix of the bone consists mainly on inorganic non-stoichiometric carbonate-containing hydroxyapatites and type-I collagen. As shown in Figure 4, the nano-structure of bone consists of apatite nano-crystals arranged on collagen fiber in roughly the same orientation. These nano-scale composite fibers are arranged in a random fashion in spongy bones. In cortical bones, they are bundled together in the shape of a sheet with all the fibers aligned in the same direction, and the sheets are stacked in mutually different orientations to form a cylinder-like structure of several centimeters in length. Blood vessels run in the center of the cylinder, and spaces between the fiber sheets are filled with osteoclasts. This unit is called an osteon. Osteons are renewed continuously; this process is called bone remodeling. Bone remodeling begins with dissolution of apatite crystals in an old or a broken bone by osteoclasts and subsequent decomposition and absorption of collagen fibers. Then, osteoblasts form new bone and fill the eroded cavities, and as the new bone ripens and osteoblastic cell changes to bone cell with time, the cylinder-like layer structure develops finally forming a new osteon. Bone remodeling also takes place in spongy bones, although osteons are not formed in this location.

Guided bone regeneration (GBR) is one of the preferred methods in dental and oral surgery, and good experimental results have been reported using polymers, metals, and their composite materials. However, owing to

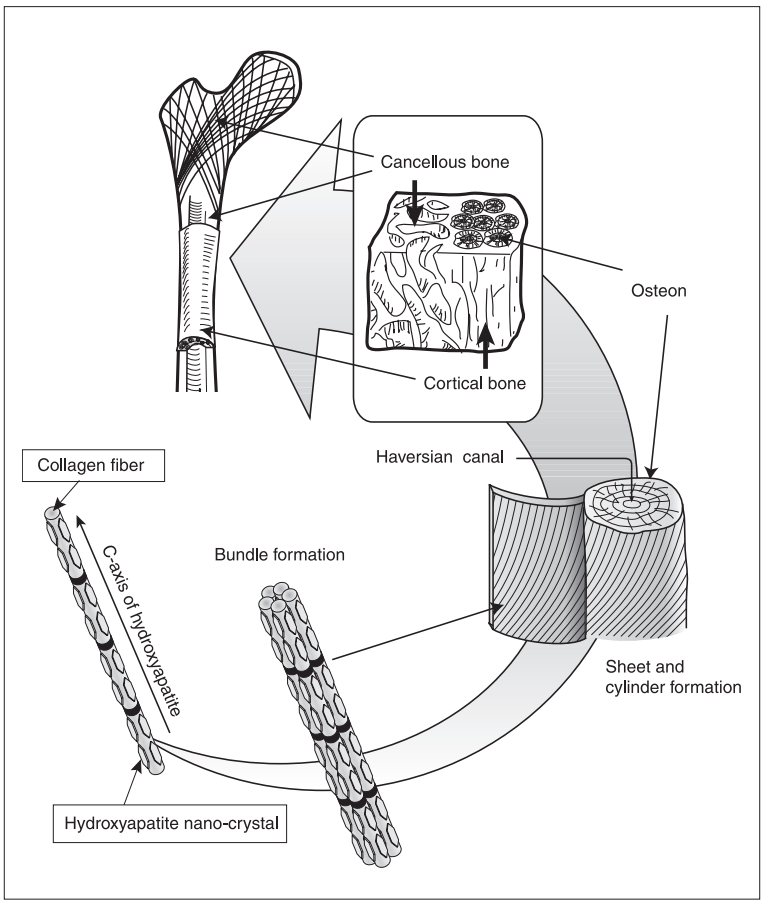

Figure 4 : Schematic view of bone structure hierarchy

the fact that non-biodegradable materials in many cases remain in the regenerated tissue, this method poses long-term uncertainties. Biodegradable materials are much more eligible for this purpose. The method that has stood in the spotlight in the last decade is composition of calcium phosphate that shows good affinity to biotissues with synthetic biodegradable polymers. The use of these composite materials in GBR was proposed around the same time in Italy, Germany, and Japan. This method has already been clinically applied in Italy, where doctors are allowed to exercise a high degree of discretion. In Japan, this is still in a research phase. Membrane materials used for GBR still have much room for improvement. Further upgrades are desired by, for example, adding growth factors and cell-stimulating factors and improving osteoconductive properties.

In a separate development, the tissue engineering method for strengthening the connection between an artificial joint and the adjacent bone has already been put to practical use in Japan. This method harvests stem cells from the patient's bone marrow mesenchymal cells that have the potential of differentiating into osteoblast cells. After separation and multiplying processes, the cultured cells are differentiated by adding cell stimulating factors and subsequently seeded onto the interface of the artificial joint (made of materials, such as alumina, that do not directly integrate with the bone) and the bone for better connection[10]. Regeneration of a finger bone using tissue engineering technique has also been reported. However none of these has succeeded in regenerating blood vessels in bone using tissue engineering methods. In the case of the regeneration of a finger bone, the regenerated bone does not function as an actual bone. Tissue engineering methods capable of restoring major bone defects are still in the research phase. To some extent for bone defects, a good cure can be obtained by autogenous bone graft, or grafting the patient's bones mixed with filling materials. Tissue engineering of the bone has two main objectives: one is to restore bone defects (complete bone defects up to the size of 3-5cm) in a relatively short period of time irrespective of patient age; the other is in vitro regeneration of the whole bone system including cartilage

and blood vessels. The former objective has an aspect of time-game with the development of better filling materials. For example, a bone-like hydroxyapatite/collagen nanocomposite, with nano-structure and chemical composition similar to the bone and with a self-organization mechanism, has an experimental achievement of near-perfect restoration of a 2 cm tibial bone defect created in a Beagle dog in three months[11]. However, unless the cell seeding technique can clearly show the promise of more than halving restoration period, bone filler technique will be used at least for younger generations*5.

In order to make the treatment period shorter than that of filler method, development of better scaffold materials is essential. These scaffolds should also be such materials that can be transformed to bone through the function of cells in the adjacent bone, because bone is rich in extracellular matrix and has to respond to mechanical stimuli just as in cartilage, and has a high turnover rate,. Organization of bone tissue starts with the primary structure at a level of nanometer and sequentially develops into higher-order structures that can reach the size of centimeters. In fact, a complex fiber structure of the size up to 75 mm has been successfully produced using soft-nanotechnology starting from a hydroxyapatite/collagen nanocomposite[12]. However, bone substitutes that mimic bones from micro- to macro-scale that contain oriented sheets of fibers and the mechanical properties of natural bones have not yet been produced. There is a hypothesis that substances such as cell stimulation factors incorporated in extracellular matrix of bone may enhance the function of osteoblasts during bone remodeling. At present, such biochemical function can be realized only by immobilizing molecules on the surface of bulk material. More sophisticated function will be needed in the future for delivery of an appropriate amount of biochemical factors at the correct timing. Regeneration of large bone tissues will require techniques to regenerate complete bone tissues including blood vessels. To solve this challenge, development of techniques to construct higher-order structure in step-wise fashion from nano to macro levels, as well as the nano structure construction method that mimics biological processes (soft nanotechnology). Techniques developed for solving these challenges are likely to have applications in the regeneration of other organs that have scarcely any extracellular matrix.

Artificial bone grafts are used in only about 20% of cases that require bone transplantation. The current market size is approximately 7 billion yen, and is forecasted to saturate roughly at the level of 10 billion yen. However, when the next generation artificial bones and tissue engineering come of age for practical applications, the expectation is that autogenous bone grafting that is highly invasive will go out of use, in which case the market size (domestic market only) will grow to the level of 50 billion yen or more. In the United States, the artificial bone market has been relatively small because the use of autogenous and heterogenous (cadaver) bone transplantation has been the dominant method (even heat-treated bovine bones are used in some cases). However, due to concerns about infectious diseases, the demand for artificial ceramic bones has increased in the last decade. All these aspects inclusive, the size of bone related tissue engineering market is likely to reach 300 billion yen. Furthermore, regenerated bones using tissue engineering are, unlike conventional artificial bones, considered applicable for a wider range of patients including infants, elderly people and even people with bone metabolism disorder, making it possible to boost the market size to the level of 600 billion yen.

3-4 Pancreas and Liver

Cells in visceral organs such as the pancreas and liver, have very little extracellular matrix: interaction between the cells plays a more important role in expressing the function of cells as a component of an organ than in skin, cartilage and bones, thus making regeneration through tissue engineering techniques more difficult. As shown in Figure 5 above, the pancreas is located beside the duodenum and its function has two aspects: as a part of the digestive system secreting pancreatic juice that contains digestive enzymes, and as a part of the endocrine system secreting insulin and other hormones. The endocrine function resides in the pancreatic islets, which

are located in the pancreas and consist of one to two million cells (See Figure 5 below).

The liver, shown in Figure 6 above, is involved in energy metabolism and detoxication and other functions that are essential to life. These functions are implemented by the cells and vascular system depicted in Figure 6 below[7].

Because regeneration of the liver and pancreas as a whole using the currently available technologies is next to impossible, the mainstream view to solve this problem is to regenerate selected minimal functional units and implant them en masse to effectively substitute the organ. For the pancreas, transplantation of the pancreatic islets has already begun for patients with insulin-dependent diabetes mellitus or patients who cannot produce insulin due to removal of pancreas because of cancer or other reasons.

Among the many attempts to regenerate functional units of these visceral organs, formation of spheroids is attracting attention and most actively under development. A spheroid is a mass of cells that are produced using non-adherent round-bottom culture plates. A variety of techniques is currently under consideration to produce high-density spheroids with controlled cell behavior. In terms of cell culture using nonadhesive plates, use of rotating wall vessel and clinostats*6 are considered promising, and many attempts are being made to produce various types of spheroids while controlling their sizes and maintaining cell functions. One notable experiment using a rotating wall vessel is reported to have succeeded in producing hepatocyte spheroids that contain blood vessels and biliary tracts starting from hepatocytes harvested from a mouse fetus[13]. Further development of this experiment is being watched closely with expectations. Many attempts have been made to produce a large amount of spheroids in non-adherent culture plates by controlling cell's adhesive properties[14]. This approach is attracting attention because it does not require special culture techniques or devices.

In parallel to production techniques, the source

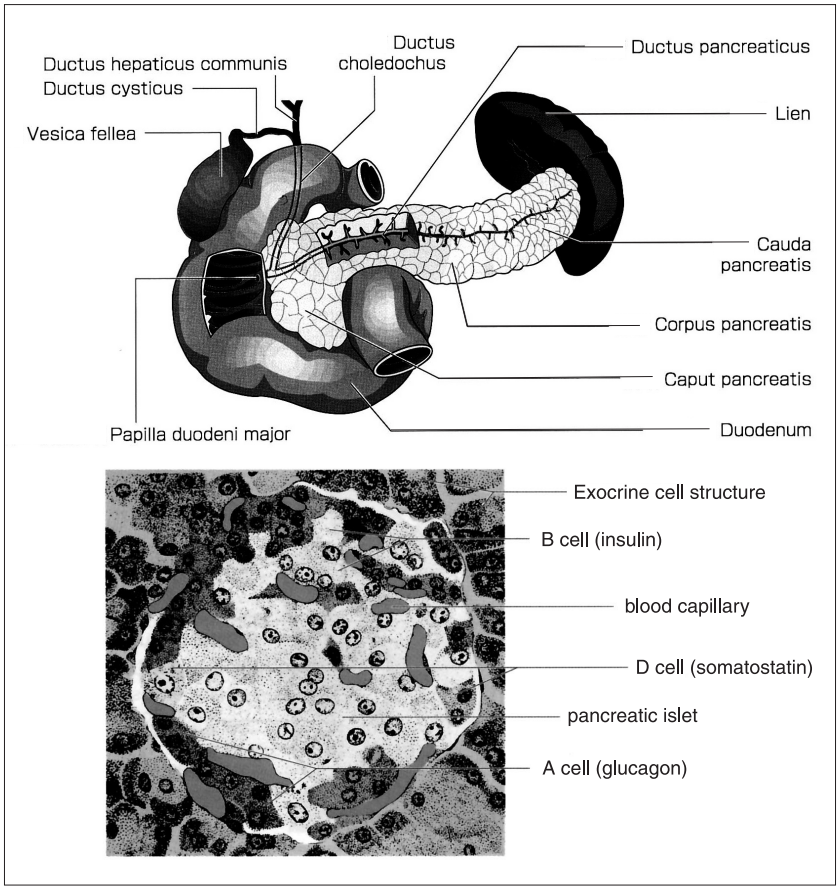

Figure 5 : Pancreas and surrounding organs

Above: Overview Below: Schematic view of pancreatic islet for insulin production
Quoted from Reference[7], p.155 (above), p.114 (below)

of cells is also an important matter to consider. In many cases, organs become necessary after the patient's own organs fail, making it necessary to either differentiate cells from the patient's own stem cells, or to culture another person's cells. In the latter case, the cells and secretion from the cells can cause immunological reactions, entailing the development of the immunoisolation method. These isolation materials have to allow necessary components to pass through while blocking immune reaction causing substances, which is quite a difficult task. For example, immunoisolation materials used for the pancreas must permeate insulin, nutrient components, and waste.

Naturally, regeneration of organs with all the original functions and adequate size is the final target. For this purpose, the highly desirable aim is the development of an auxiliary biomaterials that provide a 3-dimensional framework for initial organ development and maintains its structural integrity until the organ can hold its own shape using only the cells and extracellular substrates secreted from them.

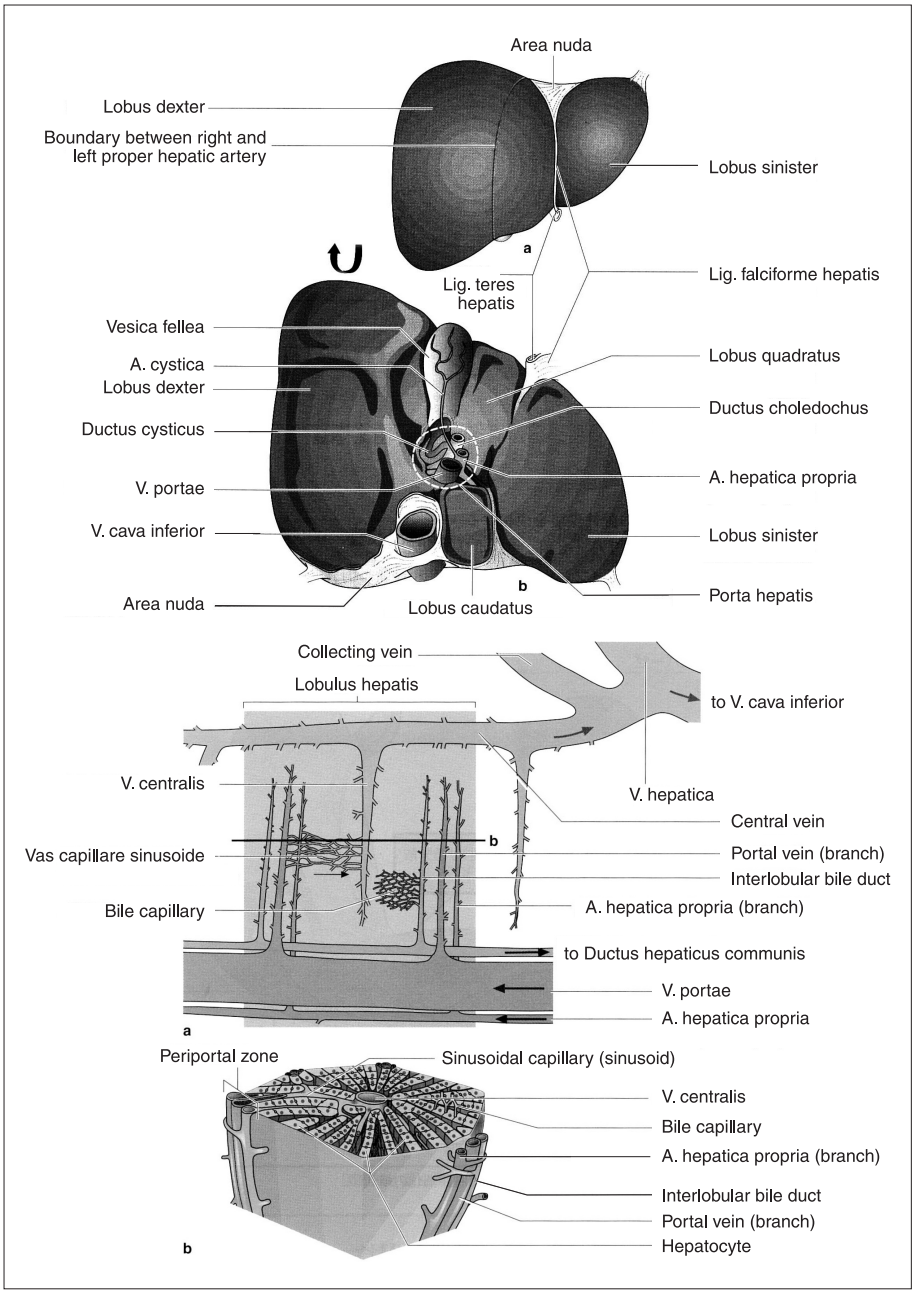

Figure 6 : Liver organization

Above : Whole image (a: front view, b: bottom view)
Below : Liver lobule (a: vertical section, b: horizontal section)
Quoted from Reference[7], p.156 (above), p.157 (below)

Let us estimate the size of the pancreas related regenerative medicine market from the number of diabetic patients. Currently, the estimated number of patients is 2.469 million in Japan, and 0.246 billion worldwide, and will reach 0.38 billion in 20 years[15]. Assuming that 10% of patients are indicated for artificial pancreas, and the cost per patient is around 3 million yen, market size would be 84.5 billion yen in Japan[8] and 850 billion yen worldwide.

Commercialization of artificial hybrid liver (combination of liver cells and biomaterials) is underway with a unit price of around 5 million yen. These units are estimated to create a 48.8 billion yen market by 2020[8]. Regenerated liver will not only supplant these artificial units, but will also increase the percentage of patients viable to liver transplantation (including the use of regenerated liver) up to 20%. There are some 40 thousand patients who need liver transplantation in Japan; thus the market size is expected to grow up to around 40 billion yen.

3-5 Challenges of tissue engineering

As described above, culture and multiplication of cells as two-dimensional structures is relatively straightforward, but to develop tissues in three-dimensional shapes retaining cell functions is extremely difficult. Okano et al. of Tokyo Women's Medical University, who are advocating cell sheet engineering, are conducting studies on the clinical application of corneal epithelial cells that can be brought into practical use with two dimensional culture[16]. However, in other applications, for example myocardial sheets, three-dimensional grafts are quite difficult unless lamination process is carried out by repeated surgeries. Cells need provision of oxygen and nutrient, and they have to dispose of waste matter. In the environment without vascular system, provision and disposal must depend on liquid phase dispersion, causing cell necrosis in the core of thick cell mass. Similar problem can occur in preparation of large spheroids and culture of osteoblasts in three-dimensional porous media. Regeneration of clean organs requires scaffolds where cells can multiply, culture media can be easily exchanged even in the core of tissue, and the vascular system can develop inside (cartilage excluded). Hitherto, efforts have been made, and to some success, to provide surface preparation on mono-functional scaffold materials for better adhesion properties, or simply adding cell stimulus factors to provide above-mentioned functionality to the scaffold. To date, no attempt to reproduce an organ fragment even of the size of 1cm has succeeded.

Taking the above into consideration, to overcome this one-centimeter barrier, it could be useful to produce tissues that perform multiple functions containing different types of cells as found in the living bodies to provide appropriate cell environment over time just as in the natural developmental process. Thus, an important agenda for material development is to produce scaffolds that allow four-dimensional control, i.e, three-dimensional control over time. Such scaffolds should provide a cell environment that can change its state synchronized to, or in advance to, the development, differentiation, and induction processes of the cells, similar to that of actual biological body.

Biodegradable metallic implant materials using magnesium alloy are regaining attention. Many attempts were made in the 1930s and 1940s to apply these alloys to orthopedic materials because they have similar Young's modulus as bones. However, these attempts subsided gradually for two main reasons: magnesium alloys can produce hydrogen that forms air bubbles around the implant and the use of stainless steel with superior mechanical properties became common in starting in the 1940s. In regenerative medicine where biodegradability is an important factor, magnesium alloys with good mechanical strength are an attractive option. In contrast, ceramics are also promising in terms of biological affinity. The expectation is that combinations of ceramics, organic polymers and magnesium alloys will help the development of better scaffold materials.

4 Points for improvement of biomaterial research and practical application

4-1 Official licensing procedures

There is fierce R&D competition taking place among nations in the field of biomaterial and tissue engineering. Developed nations are aiming at rapid introduction of these results to the medical and welfare market to cope with their aging populations. Regenerative products consist of a wide variety of valuable products in small quantities.

Our first requirement towards practical application of this research is to develop an efficient and speedy licensing system for biomaterials or medical devices, and to participate in efforts to establish international standards controlling the biomaterials and medical devices.

A long process is required before biomaterials and tissue engineering techniques can be used clinically: After confirmation of the safety and efficacy of the investigational material/technique in cell culture assays and animal studies, an application for clinical studies of the material/technique should be submitted, and then an application for approval of the material/technique containing data in human should be approved before marketing the material/technique. In Japan, cell-material composites for use in regenerative medicine are regarded as a "medical device" that contains medicinal substances, while materials used for drug delivery systems are regarded as a "drugs" (both are regarded as medical devices in the U.S.). The licensing process for medical devices in Japan is similar to those applied to drugs: a rigorous and lengthy review process is required.

Although vigilant and discreet evaluation is quite justifiable because these products involve human lives, however, applying the same review process as for the drugs includes apparently superfluous steps. Taking implantation of autologous cells for an example, physicians are allowed to harvest, multiply and transplant the cells at their own discretion on the condition that the procedures take place in the hospital to which they belongs. However, if the multiplication process of the same autologous cells takes place in a commercial facility outside the hospital, a separate official approval is required entailing tracking back to the origin of the cells. Careful pedigree evaluation of the cells is important for non-autologous cells because of infectious concerns, but it is highly questionable if these verification steps are still needed when it is apparent that the cells are autologous.

In the U.S., a straightforward supplemental application is available as a method of partial modification of the approved items of a medical device. With this method, modification of an approved medical device can be approved promptly. The same situation in Japan requires going through the whole application process again. Because of this, a case has been reported where an imported product cannot be sold in Japan due to minor modification: the distributor has to stock older parts and assemble or repair the device using the older parts to sell the device in the Japanese market[17]. These rigorous procedures are applied even to regenerative medicine and DDS materials. Efforts should be made to minimize the burden for approval for any combination of those materials, cells, and medical agents that have already been approved, unless there could be a possibility of evolving chemical reactions among them.

4-2 Conflict of views: industry and public administration

Measures towards expediting the examination period have been discussed in the Council for Science and Technology Policy, and a plan to double the number of examiners in three years has been announced, but distribution of area of expertise of these examiners has not been clarified. Joint effort between private sectors and the Ministry of Health, Labour and Welfare (MHLW) towards speed-up of examination is in progress through the activities of the Medical Engineering Technology Industrial Strategy Consortium and Regular Meeting on Pharmaceutical Regulations of Medical Equipment. Unfortunately, it seems that communication among these efforts is insufficient and we will have to wait for some

time before these efforts will deliver concrete results.

Table 2 provides a summary of opinions from industries and administrations put forward at the 5th Regular Meeting on Pharmaceutical Regulations of Medical Equipment. The industry sector hopes to carry forward clinical trials by collecting necessary information and data required for application protocol as quickly as possible. Consultation meetings can be a good tool for accelerating this process. The industry sector is generally dissatisfied with the response from the administrative side because of the lack of rational viewpoint and other reasons and feels this can be the major cause of delay in approval process. Lack of a rational viewpoint causes confusion such as hiatus of important data, delay of data processing, and other such problems, resulting in the whole process. The administrative side, in contrast, has an understanding that it has delivered a sufficient amount of explanation and advice. On the whole, opinions from both sides do not seem to mesh well including the interpretation of terminology, and might well be resulting on the administrative side stipulating more than necessary data to be on the safe side.

4-3 Participation in international standardization

Japan has traditionally paid insufficient effort for proactive international standardization. There is no denying that a passive "just follow what others decide" attitude still prevails. The U.S., for example, has a standardization organization, the American Society for Testing and Materials (ASTM) that strongly reflects U.S. industry's interests. ASTM is quite proactive in establishing international de-facto standards and actively lobbying to incorporate their interests into the International Organization for Standardization (ISO) standards.

A welcome sign is that some authorities and experts from industry and academia in Japan have started to participate in regenerative medicine related technical committees, such as on orthopedics materials and biological materials. Also, the drive for the standardization effort can be seen among Japanese researchers as evidenced by participation in the pre-standardization organization of advanced materials, Versailles Project on Advanced Materials and Standards (VAMAS), from its initial stage. Continued effort for this line of activities is strongly desired. The Ministry of Health, Labour and Welfare should introduce international standards for evaluation of medical devices. It is also important to rationalize domestic standards and propose them for international adaptation, which will surely effect to the advantage of Japanese medical devices gaining ground in the international market.

5 Conclusion

We have discussed the current status and future problems in biomaterial research focused on regenerative medicine. Outstanding points among the status and problems are as follows.

Table 2 : Difference of view between industry and administration
(at the Regular Meeting on Pharmaceutical Regulations of Medical Equipment)

Views from industry	Views from administration
• Delay of examination due to insufficient number of examiners • Irrational examination process • Disparity between regulation and development promotion • Provisions for devices approved in other countries • Relaxation of regulation on Good Clinical Practice (GCP) • Difficulty in using preapplication consultation	• Insufficient quality of application dossier • Slow flow of information from manufacturer • Insufficient compliance to what was decided
Common views	
• Drug specialists are supported by academia and industry, but there are no academic units to foster medical device specialists. • Fundamental difference of drugs and medical devices. (Takashi Wachi: Chairman, the Japan Federation of Medical Devices Associations, Tatsuo Kurokawa: Councilor (Pharmaceuticals), Minister's Secretariat, MHLW)	

Compiled by Mr. T. Tateishi (National Institute for Materials Science) based on Reference[18]

5-1 Control over time of cell environment and development of scaffold materials to support cell environment

To reproduce large functional tissues, it is essential to create and control an appropriate environment that ensures the growth of cells during regeneration and avoids necrosis. Especially, the development of such materials (or artificial extracellular matrix) that can be controlled in terms of three-dimensional structure and time so that the cell environment can be adapted synchronized to or preceding the changes in cell's functional expression is required. Regeneration of organs requires an environment in which different types of cells can function in cooperative fashion. For this purpose, appropriate space must be prepared in the tissue environment for the cells to multiply, differentiate, and develop functions. Physicochemical or biochemical conditions around the organ change constantly. Future scaffold materials that should be developed must be able to control the cell environment in terms of time and should finally disappear.

5-2 Infrastructure for accelerating regenerative medicine research

Timely approval of study products is essential to promote practical application of regenerative medicine research. One of the bottlenecks toward this goal is the fact that some of the data required for examination seems to lack rational grounds, delaying the approval process of new regenerative medical products. Another deplorable problem is the conflict of views that are still persisting between industries and administrative communities. An effective measure against the delay of approval processes and for the establishment of an effective system will be to foster experts with ample knowledge in medicine and pharmaceutical science, and allocate them to authorization organizations or medical facilities. Establishing a new national qualification, corresponding for example to a pharmacist, may be a good idea. Distribution of such expert personnel is also important from the perspective of maintaining medical equipment that becomes increasingly specialized and complex and preventing medical malpractice due to erroneous instrument use. An equally important factor is the effort to establish international standards for medical and technological evaluation that is acceptable or favorable to Japan. This will have a tremendous effect on both acceleration of clinical trials and dissemination of products in the world market, thus stimulating the medical industry in charge of practical realization of research results.

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Glossary

*1 Hydroxyapatite is a main inorganic constituent of vertebrate bones and tooth. Hydroxyapatite may found as calcium-deficient hydroxyapatite, sodium hydroxyapatite, magnesium hydroxyapatite, hydroxyapatite of which carbonate group is replaced with phosphate group or hydroxyl group; and those of which hydroxyl group is replaced with fluorine.

*2 Buffer solution that has nearly equal inorganic components as human plasma. When bioactive ceramics (capable of direct connection with bones inside the body) is immersed in this fluid, fine crystals of carbonic acid containing hydroxyapatite is deposited on its surface.

*3 Most cells except blood cells and other cell types in suspension need to be fixed on matrix for multiplication and differentiation.

Cells in the body are adhered to extracellular matrix such as basal membrane. For effective in vitro multiplication and functional expression of cells, alternative extracellular matrix is required. Such matrix is called a scaffold. Currently, porous materials made of collagen, polylactic acid, and hydroxyapatite are used.

*4 Since regeneration of cartilage and surrounding bone tissues is impossible in patients with degenerative arthritis, their diseased joints are often replaced with artificial joints made of metal and ceramics. This is a viable method because the main function of cartilage is mechanical. However, prolonged use of artificial joints can cause problems in joining area with bone and sliding surfaces; hence an alternative regenerative method is highly desirable.

*5 Elderly people generally retain a considerable degree of bone formation capacity, but many of them have osteoporosis. For safety reasons, many physicians prefer materials containing osteoblasts.

*6 Rotating wall vessels are uniaxial rotating culture apparatuses used to produce a pseudo microgravity environment, which are derived from technology developed by NASA. Klinostats are also rotating culture apparatuses capable of producing even better a pseudo microgravity environment by rotating 3-dimensionally.

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