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Tissue engineered products for pediatric patients

Produkty inżynierii tkankowej dla pacjentów pediatrycznych

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S u m m a r y

Tissue Engineering, a new field of regenerative medicine, offers products for tissue regeneration obtained by means of cell culture, biomaterials and factors which stimulate tissue growth. Tissue engineered products (TEPs) are an alternative to the methods currently used for tissue regeneration, e.g. transplantation of autogenic or allogenic tissues, or implantation of synthetic materials.

The need for tissue regeneration in children results from congenital malformations, traumas or defects caused by surgical intervention, e.g. tumor resections. One of the potential advantages of tissue engineered products over classical methods for tissue regeneration in children is related to the feature that implants obtained *in vitro*, after their implantation, should participate in the process of growth of the young organism (and a rebuilding of the graft is expected to occur). Therefore, no morbidity occurs, as opposed to the implant exchange otherwise conventionally performed due to the patient's development.

There are several tissue engineered products of bone, cartilage and skin on the market already. Research is carried out on launching other tissue substitutes on the market, such as vessels, valves or pancreatic islets. Many scientific reports are published about the experimental use of tissue engineered products in pediatric patients with TEPs made from autologous cells, biomaterials and factors stimulating regeneration and growth of the reconstructed tissue.

The article presents basic information about tissue engineering, its methods and products already present on the market, followed by a description of the regeneration of skin and epidermis, pulmonary artery, bladder, urethra and bone reconstruction in children.

Key words: tissue engineering, tissue engineered products (TEPs), tissue regeneration

S t r e s z c z e n i e

Inżynieria tkankowa to nowa dziedzina medycyny regeneracyjnej, zajmująca się możliwościami regeneracji tkanek przy pomocy produktów leczniczych zawierających komórki z hodowli *in vitro*. Zwykle komórki przeznaczone do transplantacji są osadzone na podłożu biomateriałów, często z dodatkiem czynników stymulujących wzrost tkanki. Produkty inżynierii tkankowej (PIT) są alternatywą dla obecnie stosowanych metod rekonstrukcji tkanek – tj. przeszczepów autologicznych, allogenicznych lub implantów z materiałów syntetycznych.

Potrzeba regeneracji tkanek u dzieci pojawia się w przypadku niektórych wad rozwojowych. Występuje także wówczas, gdy potrzebne jest zaopatrzenie ubytków powstałych w wyniku urazu lub interwencji chirurgicznej, np. u pacjentów onkologicznych. Potencjalna przewaga produktów inżynierii tkankowej nad rozwiązaniami dostępnymi obecnie u pacjentów pediatrycznych polega między innymi na tym, że procesowi wzrastania i rozwoju młodego organizmu powinna towarzyszyć przebudowa przeszczepu. Pozwala to uniknąć wymiany przeszczepu w trakcie rozwoju pacjenta.

Dostępne są już komercyjnie produkty inżynierii tkankowej do regeneracji skóry, tkanki kostnej i chrzęstnej. Trwają prace nad wprowadzeniem na rynek kolejnych substytutów tkanek, jak np. naczyń krwionośnych, zastawek serca, wysepek trzustkowych. Wiele doniesień naukowych zawiera opis eksperymentalnego wykorzystania produktów inżynierii tkankowej u pacjentów pediatrycznych. Są one przygotowywane „na miarę" z komórek własnych pacjenta, biomateriałów i czynników stymulujących regenerację i wzrost odtwarzanej tkanki.

Artykuł zawiera podstawowe informacje o założeniach inżynierii tkankowej, wykorzystywanych metodach oraz dostępnych produktach komercyjnych. Przedstawiono też opis klinicznego zastosowania produktów u pacjentów pediatrycznych w celu regeneracji skóry i naskórka, tętnicy płucnej, plastyki pęcherza moczowego i cewki moczowej oraz rekonstrukcji kości.

Słowa kluczowe: inżynieria tkankowa, produkty inżynierii tkankowej (PIT), regeneracja tkanek

The regeneration and reconstruction of tissues and organs is a current clinical issue in pediatrics. The need to restore or augment the function of tissue applies to all organs; in children it may be caused by congenital malformations, traumas or may result from a treatment e.g. after a tumor resection (1). A variety of sizes and shapes of reconstructed tissues should be taken into account. Moreover, one should aim to achieve the ability of the graft to adjust to the child's constant growth and development in terms of the graft's structure and function.

In this context, the clinical methods of tissue reconstruction in children used currently are not fully satisfying. Three methods are applied to regenerate tissue defects: autologous tissue transplantation (e.g. parts of bone or vessels), allogenic transplantation (e.g. biostatic grafts from cadavers) or implanting synthetic materials.

An autologous transplantation is the "golden standard", as the patient's own biological materials is the best for tissue regeneration. Autologous grafts induce tissue formation of the recipient. However, using this method is restricted by the limited resources of healthy tissue in the patient, and it is not recommended in the case of young patients in the period of their growth and development. The next method is an allogenic tissue transplantation, mainly in the form of biostatic implants prepared at tissue banks. The preparation of such implants requires the usage of highly-specialized procedures, such as implant fixing and sterilization. A serological examination of the donor tissue should also be provided in order to eliminate the risk of disease transmission. Moreover, the progress of this method is limited by the too low number of tissue donors. The alternative to the autologous or allogenic transplantation is the use of synthetic biomaterials. There is a wide variety of biomaterials prepared from metals, polymers and ceramics, as well as their composites. Unfortunately, the materials do not guarantee a regeneration of the tissue, and are often not able to integrate with recipient's tissues.

There is an intense need to find alternative, innovative methods to cure tissue defects. Due to this fact, we observe fast progress of a new field of regenerative medicine, i.e. tissue engineering.

TISSUE ENGINEERING

Tissue engineering is an interdisciplinary field which links engineering, natural and medical sciences in order to obtain biological substitutes to regenerate, maintain or augment the functions of tissue (2). The developments of tissue engineering are already applied in therapy and diagnostics. The field is based on the assumption that three components are needed for tissue regeneration: scaffolds, stimulating factors, and cells (fig. 1).

The first component of a tissue engineered product is a scaffold, which may be made of a natural or synthetic biomaterial. The role of the scaffold is to fill up the tissue defect, offer mechanical support for cells, and provide a proper environment of cell proliferation and differentiation. The material of the scaffold should therefore be biocompatible, bioresorbable, and of the required mechanical properties (3). The idea is that the scaffold implanted into the defect, after enabling the cells to proliferate, should gradually degrade, and be replaced by living tissue. The scaffold may be produced from synthetic polymers, such as poly-L-lactate (PLLA), polyglicolide (PLGA), poly(ε-caprolactone) or of polymers of a natural origin, such as collagen, alg-

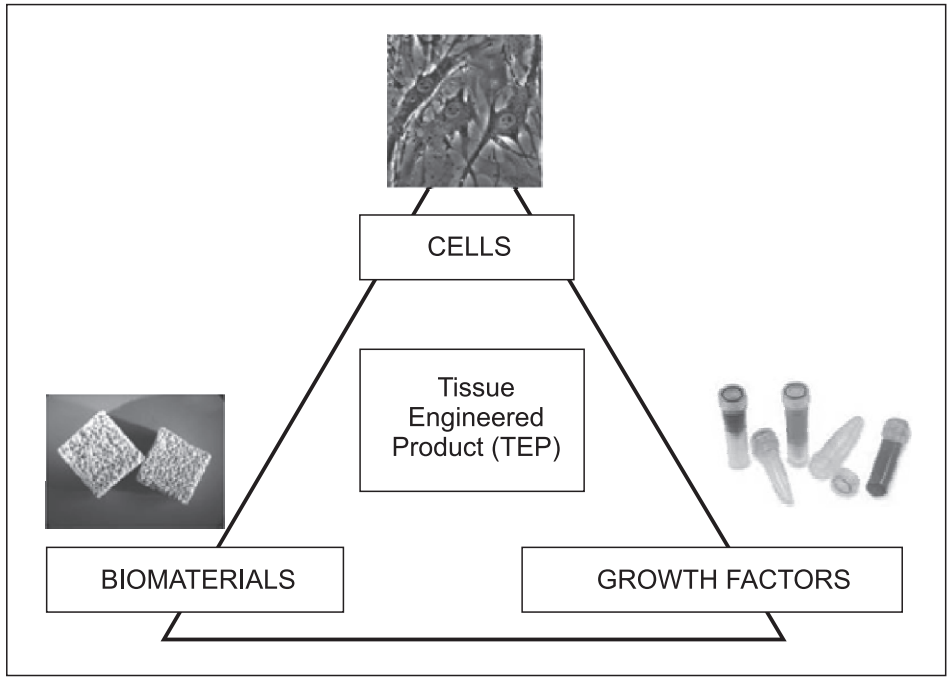

Fig. 1. Components of tissue engineered products.

inate or chitosan (4). Ceramic materials, mainly calcium phosphates, and natural bone matrix in the form of fresh autologous bone or a demineralised matrix, are also taken into account.

The second component of the triad are stimulating factors. The formation of each tissue demands a microenvironment which would provide proper factors to stimulate cell proliferation, morphogenesis and differentiation. It is proven that growth factors play a crucial role in tissue regeneration. Thus, biomaterials which contain proper growth factors have recently been produced (5-7). Besides, the scaffold may also be enriched with proteins (e.g. fibronectin, integrins), hormones or vitamins.

The last component of the product are living cells. Three main types of cells are used in tissue engineering: autologous cells (which offer immunological compatibility), allogenic cells (which may be rejected by the recipient's body) and xenogenic cells (which may be rejected, and additionally carry the risk of an animal disease transmission). Nowadays, somatic (8, 9) and embryonic stem cells (10, 11), as well as induced pluripotent cells (12, 13) are of interest here.

The preparation of cells for transplantation in the form of a tissue engineered product requires proper cell isolation from tissues obtained from the donor, which is followed by cell proliferation *in vitro* in order to multiply the cell number. The next step is to place the cells on a three-dimensional scaffold, followed by a cell culture in bioreactors, which are specialized devices for cell culture, providing a proper microenvironment and distribution of the growth factors. The construct, which is obtained in the above manner, is then transplanted into the tissue defect, where the cells build new tissue, and the biomaterial gradually degrades (fig. 2).

The advantage of using tissue engineering products results from combining the knowledge and practice of autologous and allogenic transplantation with the possibilities made available by using artificial material implants. In the case of using patient's own cells, there is no risk of immunological incompatibility, infection or disease transmission from the donor to the recipient. The tissue engineered product is autologous, but – contrary to the common practice in autologous transplantation – the amount of the patient's tissue necessary to produce the graft is very little. The amount of the biological material may be increased *in vitro,* and the product may therefore be tailored for the particular patient's needs.

Tissue engineered products, however, are not free of disadvantages. The main limitation is that, due to the fact that the product is tailor-made, it is sometimes impossible to increase the production, as the majority of the laboratory-invented products will not reach serial production. Additionally, the fact that the composition includes viable cells complicates the way the products are prepared, stored and transported. Thus, they are not "off-the-shelf" products, which means that there is a time gap between the time when the tissue is collected from the donor and the time when the product is ready to be implanted into the recipient's body. This time gap may last even several weeks, and it is impossible to provide the treatment at once.

THE NEEDS FOR TISSUE REGENERATION IN CHILDREN

There are many children's tissues whose regeneration by the means of tissue engineering would be an advantageous clinical alternative to the current methods. Not only defect regeneration, but also restoring the given organ's physiological function is possible to be provided (e.g. implanting active pancreatic islets releasing insulin).

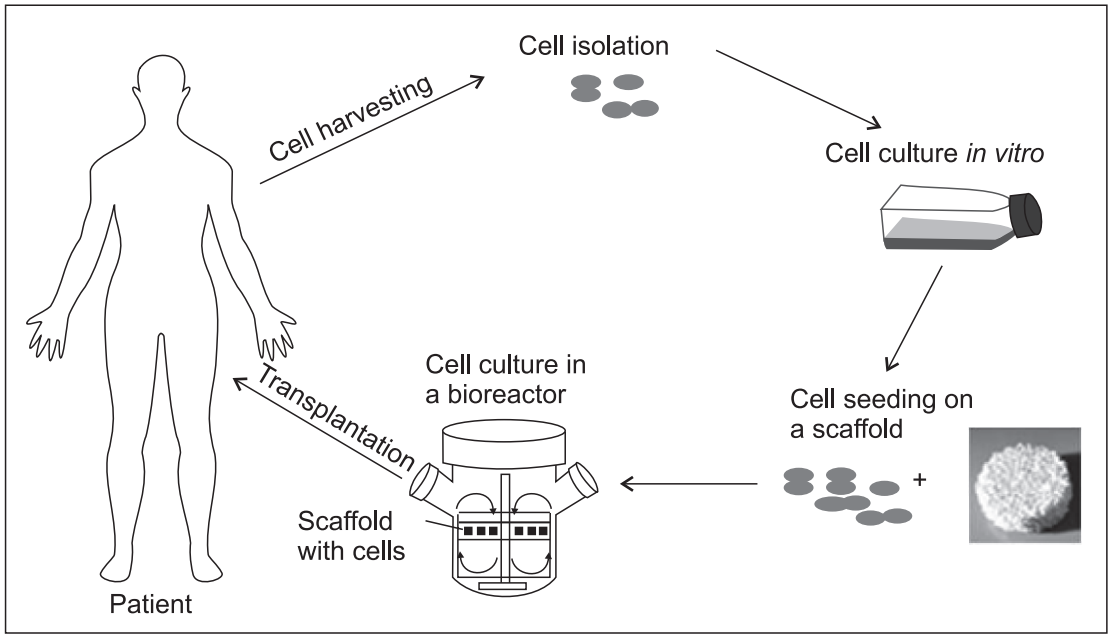

Fig. 2. The stages of preparing of a tissue engineering product (TEP).

There are several tissue substitutes for children which are of interest at the laboratories of universities or companies. Among them there are substitutes of:

- skin (necessary after burns or to reconstruct defects e.g. in the vicinity of spina bifida defects),
- bone and teeth (reconstructions of auditory ossicles, bone loss after tumor resections or after trauma),
- cartilage (e.g. in the case of chondromalacia, aseptic necrosis, to reconstruct nose cartilage or ear conch),
- tendons and ligaments (after traumas or to reconstruct tissue during orthopedic surgeries of limb malformations),
- vessels (to reconstruct congenital vessel malformations or after a trauma),
- elements of the respiratory system (to reconstruct congenital malformations of e.g. trachea or bronchia),
- liver (to reconstruct e.g. bile ducts)
- pancreas (in e.g. in diabetes – pancreatic islets releasing insulin),
- bladder, ureters and urethra (to reconstruct the organs with congenital or developmental malformations).

COMMERCIALLY AVAILABLE TISSUE ENGINEERED PRODUCTS

Considering the broadening of the commercial market of tissue engineered products, appropriate legal regulations were created. According to the regulation of the European Commission no. 1394/2007, approved by the European Parliament on 13.11.2007, a tissue engineered product is a product which is composed of cells or tissues cultured in vitro to be used to reconstruct defected tissues and organs in humans. A tissue engineered product (TEP) may consist of cells or tissues of human or animal origin. The cells or tissues may be viable or not. The tissue engineered product may also contain additional substances of cellular origin (such as cytokines, growth factors), biomaterials, chemical substances, scaffolds or matrices as a base for cells/tissues (14).

Currently there are tissue engineered products for three types of tissue already on the market, that is, for skin, cartilage and bone (tab. 1A, B, C). The majority of products are intended to regenerate skin and cartilage, while bone tissue engineered products have just started to be launched. In the case of other tissue types, the products are being tested at advanced stages of preclinical trials. Some of them, such as the tissue engineered products to be used in urology, to regenerate liver, or pancreas, have been approved to be tested in clinical trials (15, 16). Germany, the USA and Great Britain are the countries which produce most tissue engineered grafts (17). In Poland, one company, the Impomed Centrum® offers cellular therapy, based on the standards of RMS (Regenerative Medical System) to reconstruct cartilage or bone (18).

EXPERIMENTAL EXAMPLES OF CLINICAL USAGE OF TISSUE ENGINEERED PRODUCTS FOR PEDIATRIC PATIENTS

No statistical data is available which would assess the usage of tissue engineered commercial products in children. However, there are many publications which deal with the experimental use of TEPs in the regeneration of tissues in children.

Regeneration of epidermis and skin

Tissue engineered products of skin have been implanted in children after burns. An article, published in

Table 1. Tissue engineered products available on the market.

A. Tissue engineered products for skin regeneration.

Tissue engineered products for skin regeneration		
Product's name, Producer	**Composition: biomaterial, cells**	**Product's structure**
OrCel®, Ortec International Inc.	Bovine collagen type I, allogenous keratinocytes and skin fibroblasts	The cells are cultured in two separate layers: fibroblasts on the surface and inside the porous collagen sponge, keratinocytes on the other, nonporous side of the material.
Apligraf®, Organogenesis	Bovine collagen type I, human skin cells	The cells are placed in two layers: one type in the whole volume of the collagen matrix, the other one on the nonporous side of the material.
Epicel®, Genzyme Biosurgery	Gauze with petroleum jelly, human autologous keratinocytes, nonproliferating murine fibroblasts	Gause is a scaffold for cells: nonproliferating murine fibroblasts are the fedder layer for patient's own keratinocytes placed in 2-8 layers on the material's surface.
TransCyte®, Advanced BioHealing	Nylon mesh covered with porcine collagen in a silicon membrane, human newborn fibroblasts	Fibroblasts proliferating on a scaffold produce human collagen, growth factors and extracellular matrix proteins. The TEP is frozen before use, what causes cell death, but the substances produces by the cells are intact.
Laserskin®, Fidia Advanced Biopolymers	Hyaluronic acid, human autologous keratinocytes	A layer of human cells is cultured on a surface of a hyaluronic acid.
Dermagraft®, Smith & Nephew	Polyglactic mesh, human newborn foreskin fibroblasts	Fibroblasts proliferating on a scaffold produce human collagen, growth factors, extracellular matrix proteins and cytokines. 3D human skin substitute is produced. The products is kept frozen before implantation.

B. Tissue engineered products for cartilage regeneration.

Tissue engineered products for cartilage regeneration		
Product's name, Producer	**Composition: biomaterial, cells**	**Product's structure**
BioSeed®-C, BioTissue	3D biomaterial, human autologous chondrocytes	Chondrocytes are cultured *in vitro* in the whole volume of the biomaterial.
Hyalograft 3D®, Fiadia Advanced Biopolymers	Hyaluronic acid, autologous chondrocytes	Cells are places within the whole volume of the 3D scaffold made of hyaluronic acid.
Carticel®, GenzymeTissueRepair	–, autologous chondrocytes	The product is composed of chondrocytes cultred *in vitro*, the ACT (Autologous Chondrocyte Transplantation) procedure is used for implantation.
MACI®, Verigen	Bovine type I and III collagen, autologous chondrocytes	System MACI (Matrix induced Autologous Chondrocyte Implantation) is applied. Cells, multiplied *in vitro*, are seeded on the collagen membrane cut in form of a cartilage defect.
co.donChondrosphere®, co.don AG	–, autologous chondrocytes	The product is composed of chondrocytes cultured *in vitro*, the ACT (Autologous Chondrocyte Transplantation) procedure is used for implantation.
CeReS 3D®, ARS ARTHRO AG	3D collagen scaffold, autologous chondrocytes	The structure is not described by the producer.
ARTROCell®, Ormed	Porcine collagen type I and III, autologous chondrocytes.	The chondrocytes cultured *in vitro* are covered be a collagen membrane.

C. Tissue engineered products for bone regeneration.

Tissue engineered products for bone regeneration		
Product's name, Producer	**Composition: biomaterial, cells**	**Product's structure**
co.donosteotransplant® DENT, co.don AG	–, autologous ostoblasts	The product is composed of osteoblasts cultured *in vitro*, the AOT (Autologous Osteoblasts Transplantation) procedure is used for implantation.
Osteocel Plus®, NuVasive	Spongy bone and demineralized bone matrix, mesenchymal stem cells	The structure is not described by the producer. The product was approved by the FDA for clinical use, but not as a tissue engineered product, what was linked with less demanding application procedure.
Ossron®, RMS Regrow	Biodegradable gel, autologous osteoblasts	The mixture of cells and gel is injected into the defect site.

2005 in Lancet, describes a successful implantation of skin substitutes obtained by the means of tissue engineering in 8 patients at the ages of 14 months up to 9 years (19). The cells were grafted within horse collagen matrices. They were isolated from a piece of a fetal skin. According to the authors, a 4 cm^2 piece of the skin surface, after the isolated cells were multiplied *in vitro*, enabled them to create a cell bank to be used for several millions of such products of the dimensions of 9 x 12 cm. Although the cells used to obtain the skin substitutes come from an allogenic donor, it is stated that cells of fetal origin represent a lower expression of MHC proteins. Total wound closure with a little of tissue hypertrophy was reached after 15 days on average. No restriction of the wound edges was observed, which is an advantage in comparison to the result of an autologous skin transplantation, which is regarded as a "golden standard". Attention was paid to the excellent surgical handling of the substitute, which could easily be placed even in anatomically complicated sites, such as in the regions of toes. The authors also present an advantage of the tissue engineered substitutes obtained from the cells from the cell bank, as the two-step procedure was not needed, and the patients did not have to wait for the graft. In the cited study, male cells were used, which enabled to "track" the cells by assessing the Y chromosome in the female recipients after the implantation. An interesting result was that all of the implanted cells were absent from the graft after 6 months. The site was filled only with the recipient's cells.

After a year, in 2006, research results were published where autogenous skin substitutes composed of two cell types were used, i.e. fibroblasts and epidermal cells obtained from skin biopsies (20). The isolated fibroblasts, after a proliferation *in vitro*, were placed in a culture in a human clot. Keratinocytes were seeded on the top surface of the construct and cultured further up to 26 days. The patients who obtained such skin substitutes were aged 8-18, and had burns or skin malformations due to neurofibromatosis. It is worth mentioning that the grafts were produced in a laboratory, and then transported to clinics for long distances (up to 500 km), which did not negatively influence the results of tissue reconstruction. In all of the cases, a total regeneration of the epidermis was observed.

Bladder and urethra reconstructions

In 2006, in Lancet, research results were published, where bladder reconstructions using autologous grafts obtained by tissue engineering of muscle and epithe-

lial cells were performed in children (21). The cells were obtained from bladder biopsies. Then, they were placed in collagen and polyglicolide matrices. After 7-8 weeks, the products in the shape of a bladder of a 150 cm^3 volume were ready for 7 pediatric patients. As opposed to the traditional method of bladder reconstruction, which uses the tissues of the gastrointestinal tract, fully functional organs in terms of the mechanics and metabolism were obtained. The proper functioning of the bladder was assessed 46 months after the surgery.

The results of urethra reconstruction using two types of autologous cells were published by the same research team (22). The scaffold which was used was prepared from polylactate and glycolide. The whole procedure lasted from 4 to 7 weeks. Five boys who had 4-6 cm long urethra defects were recruited to the trial. No complications were observed just after the surgery – nor infections or fistulas during the next observation time points. Biopsies were taken at various times in order to check the quality of the grafts. Proper histological structure of the grafts was confirmed. Functional assays of the urethras indicated their proper functioning.

Reconstruction of the pulmonary artery

Methods of tissue engineering were used to reconstruct a 2-centimeter long piece of a pulmonary artery in a 4-year old girl, after an artery obliteration caused by an earlier Fontan surgery (23). The cells used for preparing the construct were isolated from the peripheral vein. The cells were let to proliferate *in vitro* for 8 weeks, and then were placed in a synthetic, degradable scaffold composed of polycaprolactone and polylactate. The graft was functional even 7 months after surgery; there were no occlusions nor aneurysms observed. Such procedures were then performed successfully in three other patients (22). The authors stress the fact that especially in the case of vessel and valve reconstruction, the use of biological grafts, as opposed to synthetic materials, guarantee the growth of the implant alongside with the growth and development of the child. Additionally, when the material used is covered by cells, no long-lasting therapy with antithrombotic drugs is necessary (22, 24).

Bone reconstruction

Patients' own cells and biomaterials were used to regenerate bone tissue in children in several clinical trials. In 2001, tissue engineered products were used to regenerate a 4-centimeter long tibia defect, after unsuccessful conventional treatment. The defect site was filled with hydroxyapatite, seeded with mesenchymal stem cells isolated from bone marrow of the patient (25). An observation after a few months and after 7 years revealed a total integration of the implanted graft.

Successful autologous regeneration of bone tissues performed by the means of tissue engineering was used in 2006 to reconstruct a bone defect of the palate in a 9-year-old girl (26). Mesenchymal stem cells, isolated from bone marrow and cultured in vitro, were injected with platelet-rich plasma into the defect site. Serial tomography visualized a gradual regeneration of the defect, and proper teeth growth was observed. In another case, mesenchymal stem cells, placed in a 14-year-old boy's autologous jaw, resected due to hemangioma, served to reconstruct the 15-centimeter-long bone defect. (27).

It is worth mentioning that all the above studies on regeneration used mesenchymal stem cells isolated from bone marrow. These cells are able to differentiate toward osteoblasts. The harvesting of the cells is less invasive than resecting a piece of bone to isolate osteoblastic cells. In two above cases, injectable materials were used to lessen the morbidity at the implantation site.

CONCLUSIONS

Tissue engineering offers a new possibility to reconstruct and regenerate defected tissues in children. Tissue engineered products composed of biomaterials and cells are already available on the market. They serve to reconstruct skin, catrilage and bone. Research is being performed on commercializing products for the regeneration of other tissues and organs. Various results of successful implantation of "tailored" tissue engineered products to regenerate tissues and augment tissue function in children are being published.

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