THE ROLE OF TISSUE ENGINEERING IN THE TREATMENT OF BURN WOUNDS

WOJCIECH ŁABUŚ¹, MAREK KAWECKI¹,², MARIUSZ NOWAK¹

Centre For Burn Treatment in Siemianowice Śąskie
Kierownik: dr n. med. M. Nowak

Department of Health Science, University of Technology and Humanities in Bielsko-Biała²
Kierownik: prof. dr hab. inż. R. Barcik

Thermal injuries may cause significant damage to large areas of the skin. Extensive and deep burn wounds require specialistic therapy. The optimal method in the strategy of treating extensive, full thickness burns (III°) is the use of autologous free skin grafts of intermediate thickness (1-4). The main limitation of this method is the inadequate amount of healthy, undamaged skin (donor site), which could be used as a graft. Moreover, donor sites are an additional wound (1, 5). The areas from which the skin samples were collected for transplantation require analgesic therapy, leaving scars during the healing process (6). The above-mentioned necessitates the search for new treatment methods considering severe burn wounds, and preparations which could effectively act as skin substitutes.

Biomaterial engineering as a promising strategy in the management of burn wounds

An attempt to define the term „biomaterials” has been undertaken repeatedly. The term refers to a single or composite substance both, natural or synthetic, which may be applied as a graft, in order to improve the functionality of living organs and tissues (7, 8, 9).

An alternative method of burn wound therapy

An alternative life-saving procedure in the treatment of extensive, full-thickness skin burn wounds with donor site deficits consists in the use of in vitro cultures of autologous keratinocytes or bioengineered skin substitutes (biological dressings) (10-13). The clinical use of skin substitutes is intended to protect the microenvironment of the wound from the adverse effects of the external environment, enabling rapid recovery of integument continuity. Skin substitutes are expected to show good adhesion to the wound, resemble normal skin as to the permeability of vapor water and heat, being characterized by relevant mechanical properties (strength and flexibility). Additionally, they should constitute a barrier against microorganisms, be safe to use, lack the risk of infectious diseases transmission, should be non-immunogenic and non-toxic, easy to use, and biodegradable with the possibility of remodeling, which supports natural tissue reconstruction (1, 12).

Despite the large number of biomaterials obtained by means of tissue engineering, none of the commercially available skin substitutes does not possess all the above-mentioned properties and cannot fully replace the functional and anatomical role of natural skin (14).

Due to the anatomical structure skin substitutes may be divided into the following: containing the dermis and epidermis, only the epidermis, and those containing only the dermis. Due to the stability of the wound cover one may distinguish permanent, semi-permanent, and temporary skin substitutes. Considering the type of the biomaterial one may...
distinguish biological skin equivalents, such as autologous, allogenic, xenogenic and synthetic, or synthetic, such as biodegradable and non-biodegradable. Considering the presence of the cellular component there exist skin substitutes, both acellular and containing living cells. The last criterion of the division of skin substitutes considers whether the biomaterial was cultured by cells under \textit{in vivo} or \textit{in vitro} conditions (6, 10, 11, 12).

Biomaterials of synthetic or natural origin

Synthetic biomaterials are non-infectious and widely available. The limitation of their clinical use is the frequent lack of biocompatibility and triggering of an immunological response against the graft (15). The problem of the adoption of the synthetic graft, its remodeling, and long-lasting stability in the body of the recipient remain controversial issues (16, 17).

Matrices of biological origin are used to reduce the unfavorable reactions associated with the introduction of synthetic material into the recipient organism, mainly insufficient adhesion to the wound, fistula and seroma development, gradual degradation, and chronic inflammation (18). Materials of biological origin contain components of extracellular matrix, which satisfy conditions of a natural environment, providing a matrix for all cells. Additionally, biomedical materials of natural origin are characterized by reduced stimulation of the inflammatory process (19). As an important advantage of natural biomaterials one should consider their physiological activity. Additionally, the above-mentioned show similar mechanical properties to that of biological tissues (8, 20).

Biological biomaterials also exhibit many disadvantages: possibility of viral transfection, antigenicity, limited availability, and deteriorating quality of the biomaterial during long-term presence inside the recipients’ organism. However, biological biomaterials are characterized by specific properties, which are not observed in case of synthetic biomaterial (8).

Connective tissue is a rich source of collagen biomaterials

The term connective tissue is a historical term that describes the various components of living tissues, which include cells associated with large amounts of extracellular matrix components (ECM), functioning as a support for the stroma. The original group of connective tissues included the following: bone, fibrous tissue, cartilage, tendons, bone marrow, blood, and fat tissue. Stromal tissue seems to be a more appropriate name for connective tissue (21).

Connective tissue cells which include fibroblasts, chondrocytes, osteoblasts, myofibroblasts, and fat cells may be observed within the extracellular matrix elements. The above-mentioned substance has many functions comprising three major groups of biological elements. The first group consists of structural proteins, such as collagen and elastin. The second group consists of specific proteins, such as fibronectin and laminin through which the extracellular matrix ensures the tensile strength of the material and creates an environment for structural elements. The third group consists of glycosaminoglycans, which are composed of long chains of repeating disaccharide units. Together with core proteins they form proteoglycans (22). By preserving the biological properties of connective tissue elements it is possible to use these tissues in biomaterial engineering.

Tissues rich in collagen, being the source of biomaterials include the following: pericardium, heart valves, demineralized bone, bowels, cornea, skin, umbilical cord, and dura mater (7).

Removal of cells from tissues as a method of biomaterial engineering

In order to improve the properties of grafts and prolong their life, numerous biomaterial engineering techniques are used whose greatest achievement was the introduction of methods aimed at removing cells from tissues. Using these methods one seeks to obtain a non-immunogenic graft, which after transplantation, would be colonized (\textit{in vivo}) by recipient cells (23, 24).

Methods consisting in the removal of cells from tissue lead to the isolation of extracellular matrix structures (ECM). The extracellular matrix is composed of proteins, glycosaminoglycans (GAG), proteoglycans, and growth factor. ECM is considered as a biological bioprosthesis, which structurally rein-
forces the damaged tissues. Various tissues were subject to the process of cellular removal, including the intestinal, esophageal, gall-bladder, placental, pericardial, heart valve, and dermal submucosa (25-33).

Since the immune response is mainly directed against cellular membrane proteins, the removal of cells is a promising method, aimed at inhibiting the immune response of the patient after graft implantation (34).

The process of cell removal consists in chemical, enzymatic, and mechanical elimination techniques. Leaving damaged compartments after tissue processing leads towards the process of calcification and immunological reactions inside the recipients' body. It is worth noting that tissues that lack cells show natural mechanical properties, and are subject to internal remodeling, due to re-epithelialization. Thanks to these properties natural biomaterials undergo transformation in the recipients' body becoming an integral part of it (35).

Acellular skin substitutes

Acellular Dermal Matrix (ADM) or Acellular Dermal Graft (ADG) act as a scaffold that stimulates the body to initiate the regeneration-repair mechanisms (36). They are obtained as a result of the staged operation of proteolytic enzymes on allogenic skin derived from cadavers (37).

Such dressings reduce cicatrisation and deformation of healing wounds. The main factor determining the clinical success of acellular skin substitutes is the lack of cytotoxicity. The cytotoxicity of these materials depends on the method used to remove the cells. The often described processing procedure of the dermis is a two-staged method of enzymatic digestion of cellular components and crosslinking structures of the ECM by means of glutaraldehyde (in order to cover the residual cellular structures). As a result of glutaraldehyde use one may observe damage to the integrity of collagen, which adversely affects the characteristics of the graft and its toxicity (38-40). Therefore, during the prepa-

ration of acellular skin substitutes one resigns from the stabilization stage by means of the above-mentioned agents (glutaraldehyde) (41, 42).

Tissue defects healed by means of skin substitutes constitute tissue with insufficient functional properties that do not match those of natural, undamaged tissue. This problem is a vast field for further research and improvements. Currently ongoing research is focused on the development of a treatment strategy aimed at combining cultured in vitro skin equivalents, such as keratinocytes, with the matrix derived from allogenic dermis. The clinical success of the project is aimed at obtaining a living skin substitute (42).

Alloderm is a widely described, commercially available skin substitute, devoid of cells.

Alloderm is a biological dressing made from human skin, which is available in the freeze-dried form. It is a matrix devoid of cells and epidermis obtained from natural skin. Alloderm has an intact basilemma (43, 44), being easily incorporated in the environment of the wound without rejection, and does not result in an inflammatory response (14). The overall objective when using Alloderm is to minimize the contraction of tendons, the development of post-burn scars, and maintain the physiological functioning in the areas of the body which comprise the tendons (neck, shoulders, elbow, knee, ankle, wrist, and hand (43, 44).

CONCLUSIONS

Scientific achievements of recent years demonstrate that tissue engineering seems to be a useful method, considering regenerative medicine. The skin substitutes obtained by means of tissue engineering provide many possibilities considering treatment of burn wounds. Despite the many advantages, none of the available skin substitutes are the golden standard, which would be able to replace the physiological function of natural skin. This inspires further investigations.

REFERENCES