Novel perspectives on the role of the human microbiota in regenerative medicine and surgery (Review)

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Abstract. Plastic surgery is transitioning from a fine craftsmanship to a regenerative science. In wound healing, the role of microorganisms is no longer considered to be just counteracting, but also promoting. Furthermore, host-microbe interactions are essential for numerous aspects of normal mammalian physiology, from metabolic activity to immune homeostasis. Each area of the human body hosts a unique microbial community, and the composition of microbiota is dependent on the host, age and the anatomical area, and it changes according to the characteristics of the microenvironment. Every squared centimeter of skin contains ~1 billion bacteria. The majority of microorganisms of the skin are commensal or temporary passing members. Skin flora mechanisms interacting or influencing the human physical skin barrier are not well defined. Resident skin bacteria provide the first line of defence against potentially dangerous pathogens and produce small molecules that influence their microbial neighbours. Furthermore, the microbiota activates and assists innate immunity and influences adaptive immunity. Various types of immune and non-immune cells contribute to wound healing. The proliferative phase of wound healing is inversely proportional to the extent of the post-traumatic inflammatory reaction. Topical bacterial lipopolysaccharide application markedly affects wound healing by accelerating the resolution of inflammation, increasing macrophage infiltration, enhancing collagen synthesis and altering the secretion of mediators involved in skin regeneration. Various studies have investigated the biological contents of thermal spring waters, and their anti-inflammatory and immune protective roles. In addition, the regenerative properties of thermal spring waters were analysed in an experimental animal wound model. The areas treated with thermal water healed faster than the areas treated with conventional dressings, and exhibited a collagen and elastic fiber network comparable with the normal skin. Thus, the microbial environment may be considered as a potential tool in regenerative medicine and surgery.

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Contents

1. Introduction

Tissue regeneration is a fascinating subject, which has always attracted the attention of scientists. As yet the underlying mechanisms of prolonging the existence of complex organisms (by restoring the cells destroyed by trauma or damaged by diseases and aging) are not completely understood. Amongst the medical specialties, plastic surgery is predominantly concerned with investigating tissue repair, which represents the basis of a positive outcome of any surgery. In addition, advanced knowledge of anatomy and biology has resulted in increased understanding of plastic surgery. Thus, plastic surgery is currently transitioning from a fine craftsmanship to a molecular and regenerative type of medicine.

2. From reparative to regenerative surgery

The aims of plastic surgery are the repair of anatomical defects, and restoration of the functions and appearance of the body. These goals are traditionally achieved with the use of

autologous tissues. In current, daily practice autologous tissue transplantation is the only realistic choice, as homologous tissue transplantation is burdened by the hazardous side effect of lifelong, severe immunosuppressive treatment.

In recent years the term 'Regenerative Medicine' has been introduced, defined as an emerging interdisciplinary field of research and clinical applications focused on the repair, replacement or regeneration of cells, tissues or organs (1). Regenerative medicine was established as the combination of various technological approaches, which includes, but is not limited to, the use of soluble molecules, gene therapy, stem cell transplantation, tissue engineering and the reprogramming of cell and tissue types (1-3).

Unlike the animals on the lowest branches of the phylogenetic tree, in humans true spontaneous regeneration only occurs in early fetal life, where the wound healing process produces normal tissue rather then a scar (4,5). Although the primary contribution to fetal regeneration has been associated with the environment (6,7), other studies are demonstrating that intrinsic factors are critical; therefore, fibroblasts are currently the main target of studies regarding skin regeneration (5,8,9).

As a result of these novel concepts, plastic surgery is progressively overcoming limitations, and increasingly becoming integrated with other disciplines and sciences. The performance of plastic surgery is becoming more prevalent, particularly for elderly individuals who are affected by severe comorbidities and require extended surgical procedures. Therefore, regenerative plastic surgery is becoming more feasible for challenging patients (10). Regenerative medicine and surgery is characterized by three major fields; tissue engineering, biomaterials/biomolecules and stem cell therapy.

3. Microbiota and the National Institutes of Health Human Microbiome Project

Multiple factors are involved in the wound healing process and the significant role of microorganisms is well known. In the past, the influence of microorganisms was investigated only during pathologic events; however, the growing interest in microbiology and the availability of novel technologies allow greater analysis of their association with the host. Microorganisms are present in every type of environmental niche and interplay with all its components.

The term microbiota is used in the current study to define the microbial community hosted on the surface of the human body, which is composed of bacteria, viruses, fungi and archei, and the term microbiome for the whole genome of the community (11). Microbial cells living on the surface of the human body outnumber the totality of cells of our organism by a factor of ten, and the genes of those microbial cells outnumber the human genes by a factor of hundred (12). Host-microbe interactions are essential for various aspects of normal mammalian physiology, ranging from metabolic activity to immune homeostasis (13-19). Every area of the human body hosts a unique microbial community. The transmigration of microorganisms from one community to another, (due to, for example, environmental factors, genetic variations, life styles, hygiene habits and immunity) is associated with different pathologies. Furthermore, the microbial community is ruled by specific microbial communications, cell signalling, metabolic interactions and quorum sensing (20). Therefore the association between the human organism and the microbiota might be considered as a 'superorganism' (21).

From 2008, starting with the U.S.A. Human Microbiome Project (HMP), different studies were established to establish a database of the human microbiome in order to demonstrate any possible associations between the microbiota, and health and disease conditions (22-24). However, microbiota characterization is hampered by the difficulty to recreate the same conditions *in vitro* as *in vivo* (25). With the aid of current metagenomics techniques it is possible to screen the whole genes of an environmental sample and to create a genetic library of the proteins expressed by the community (26). Moving from conventional cultures to metagenomics, a prospective change is occurring, focusing on the activity and products of a whole community, rather than on a single community member (27).

4. Skin microbiota

Skin is the first defense line of the human body and houses different populations of microorganisms. The acquisition of its microbiota begins at birth (28), with the transfer of maternal microbiota, and differs between vaginal and caesarian delivery. The skin microbiota evolves over the years according to the changes of skin structure and functions (29) becoming similar to that of adults by the age of 12-18 months (30). In addition, individuals acquire their own microbiota through contact with other individuals, visiting different places, and eating food. Furthermore, microbiota composition changes according to ethnicity, geography and life style (12). Every squared centimeter of skin (including hair follicles and sebaceous glands) contains ~1 billion bacteria (31).

The majority of the microorganisms of the skin microbiota are commensal or just temporary passing members. The following four bacterial phyla are present on human skin: Actinobacteria, Proteobacteria, Firmicutes and Bacteroidetes. *Staphylococcus*, *Propionibacterium* and *Corynebacterium* (12) are the dominant genera, and are constants in interindividual distribution. Differences from host-to-host are due to the less represented bacteria. In addition, in normal human skin, there are viruses (human papillomavirus, human polyomavirus, circovirus and bacteriophages) and eucaryotes microbes (fungi and protists) (32).

The composition of microbiota is dependent on the host, their age and the anatomical area, and it changes according to the characteristics of the microenvironment, which is regulated by skin adnexa (12). Microbiota α-diversity expresses the difference in community composition, comparing a specific area of the body to other areas of the same individual. Microbiota β-diversity expresses the difference in community composition comparing a specific area of the body between different individuals. Notably, antecubital fossae have the highest β-diversity, but the lowest α-diversity (33). Considering the community composition in different individuals, it is possible to state that the microbiota of an individual is as unique as a fingerprint (31,33-38).

Metagenomic techniques demonstrate bacterial DNA deep in the dermis, although such a technology may not assess the viability of the associated microorganisms (12,32). Furthermore, it is well established that microorganisms do not

have to be alive to exert their influence on the host immune system (39-45). However, it is possible that certain microbes do survive below the epithelial barrier, but cannot be successfully cultured by current techniques. In addition, the microbiota resident on the skin surface may be translocated to a subepidermal level by phagocytic cells . Epidermal physical barriers or antimicrobial peptides (AMPs) may serve as key regulators in the maintenance of dermal microbiome homeostasis, and bacterial activity to counteract the host immune response may also contribute to dermal microbiota development (46). The skin is an active immune organ where keratinocytes can no longer be considered as the sole barrier against the external environment, although they must be considered as active components of the immunoregulatory network within the external environment, the resident cutaneous immune system and the microbiota (47). Skin flora mechanisms interacting or influencing the human physical skin barrier remain poorly defined (48). Resident skin bacteria provide the first line of defence against potentially dangerous pathogens, and produce small molecules that influence the growth and behaviour of their microbial neighbours. It is important to acknowledge that skin bacteria act in synergy and in opposition to the immune system (49).

The microbiota activates and assists innate immunity, as well as influences adaptive immunity, although these complex interactions are not completely understood (49-60). As skin microbiota is important in the development of a well functioning immune system and in the modulation of the inflammatory processes, it may be significant in the wound healing process and serve as a protective factor against cancer development.

5. Human microbiota in health and diseases

Current concepts regarding the interactions at a molecular level between a co-evolved microbiota and the host (13-19) suggest a complete re-examination of human physiology and immunology (61). The reciprocal, beneficial association between the host and the microbiota begins immediately after birth; various studies state that the microbiota of newborns differ markedly according to the delivery mode. In the case of vaginal delivery, it resembles the vaginal microbiota; however, if a caesarean delivery occurs, the microbiota resembles the skin microbiota. Recent studies demonstrated that birth by caesarean delivery is associated with an increased risk of developing metabolic and immune diseases (62). For this reason, the practice of vaginal seeding has recently been proposed; it consists of transferring the mother's birth canal microbiota onto the skin of the newborn using a vaginal swab. This practice may integrate the microbiota of infants and decrease their risk of developing metabolic and immune diseases later in life (63).

Currently, the gut microbiota are the most extensively investigated. A change in gut homeostasis is associated with the onset of various severe pathological conditions, including inflammatory bowel disease (IBD) (64), a chronic relapsing inflammatory condition comprised of two clinically and morphologically different entities: Ulcerative colitis and Crohn's disease. Although the etiology of IBD is unknown, the predominant hypothesis suggests that inflammation results from a sustained immune response towards altered or pathogenic microbiota within a genetically susceptible host (65).

A successful example of microbiota transfer as a therapeutic tool against dysbiosis (an imbalance between beneficial and potentially harmful bacteria) (66) has been accomplished through fecal transplantation in patients affected by recurrent enteric *Clostridium difficile* infections. The microbiota of healthy donor feces reconstitutes the compromised intestinal microbiota in a substantial and durable way (67). The role of dysbiosis has been advocated in a wide range of diseases, including IBD and asthma, diabetes mellitus type 1, obesity, cardiovascular diseases, insulin-resistance, dyslipidemia and psychiatric conditions (13,68-83).

Recent studies indicate that the synergy between the skin microbiota and the local immune system controls the homeostasis of the complex epithelial barrier (84). The major innate mechanism of the antimicrobial defence of the skin consists of AMPs, such as defensins, cathelicidin LL-37 and dermcidin (85). These peptides are emerging as important tools in the control of skin pathogenic bacteria. Skin lesions caused by atopic dermatitis (AD) present lower levels of various AMPs than normal skin, contributing to an increased susceptibility to infections (86,87). In addition, current studies have identified the presence of certain polymorphisms in Toll-like receptors (TLRs) or TLR signalling molecules in patients with AD. Furthermore, the skin microbiota influences the immune system, through a promoting effect on T cell response, controlling nuclear factor-κB signaling and the production of cytokines, such as tumor necrosis factor (TNF)-α and interleukin-1β (88).

Germ-free mice without commensal skin microbes have been demonstrated to produce abnormal cytokine and cutaneous T-cell populations, as they were unable to mount an appropriate immune response against the intradermal Leishmania major infection; immunity could be rescued by allowing *Staphylococcus epidermidis* colonization on the mouse skin (89). The consensus on the role of microbiota in skin diseases led to an international project, Microbes in Allergy and Autoimmunity Related to the Skin, the aim of which is to identify the microorganisms triggering and countering allergy and autoimmune diseases (90).

6. The future of regenerative surgery

Many types of immune and non-immune cells, including macrophages, neutrophils, platelets, fibroblasts, vascular endothelial cells and keratinocytes contribute to wound healing. The inflammatory response begins immediately upon injury and leads to the secretion of a variety of growth factors and cytokines, which regulate the cellular and tissue movements that are required for repair (91,92). It has been demonstrated that the proliferative phase of wound healing is inversely proportional to the quantity of inflammatory post-traumatic reaction (93). Topical bacterial lipopolysaccharide treatment markedly affects the wound healing process by accelerating the resolution of inflammation, increasing macrophage infiltration, enhancing collagen synthesis and altering the secretion of numerous mediators involved in the skin regeneration process (94); all of these effects demonstrate the role of microorganisms in the modulation of the inflammatory response.

Inoculation with *Pseudomonas aeruginosa* was demonstrated to accelerate re-epithelialization and neovascularization

in wound tissues. Neutrophil infiltration, which actively contributes to the wound healing processes, was also promoted by this bacterium through the production of TNF-α (95). It has long been known that the healing of skin irritations and skin lesions can be boosted by topical applications of spring waters where a rich presence of non-pathogenic microflora is established (96-100). In recent years, research programs have been developed to investigate the biological contents of spring waters, and their anti-inflammatory and immune protective roles. The incubation of human keratinocytes with I-modulia, a biological extract from cultures of *Aquaphilus dolomiae*, showed an upregulation of the innate immune response (101). *Aquaphilus dolomiae* is a non-spore forming bacterium belonging to the Neisseriaceae family, which is isolated from Avène thermal Water (France), historically used in the management of chronic inflammatory skin diseases. Similarly, the lysate of *Vitreoscilla filiformis* (VF) has been shown to enhance skin defence mechanisms (102) and to decrease UV-induced sunburn cells in human skin, possibly by the activation of cutaneous regulatory T cells (103). VF is a filamentous Gram-negative aerobic bacterium belonging to the Neisseriaceae family found in LaRoche-Posay thermal water, historically applied to manage chronic inflammatory skin diseases (103-105).

The regenerative properties of an Italian spring water (Comano, TN) were also assessed in an experimental animal wound model. The areas treated with this water healed faster than the areas treated with conventional medical dressings, and demonstrated a network of collagen and elastic fibers that was comparable with the normal skin (99). This spring water was then analyzed at the microbial level and a total of nine non-pathogenic different strains were isolated, which are likely to produce molecular mediators with a role in the wound healing process (100). Thus, the role of microbiota in health maintenance appears to be of paramount importance and, as a consequence, indicates towards a hypothesis of exerting a positive effect in the skin healing processes. At the microbial level, the distinction between human health and disease is important only as far as it affects the microbial condition. Ignoring the association between host and microbiota in therapeutical planning is a shortsighted conduct, as demonstrated by the spread of antibiotic-resistant microorganisms (106-111).

7. Conclusion

The mechanisms upon which the microbial community structure and the association between host and symbiont are based must become incorporated into the current definition of human health. Medical intervention must aim to minimize or avoid damage to health-associated homeostasis between humans and their microbiota; therefore the therapeutic strategies to maintain healthy skin may require the inhibition of the growth of pathogenic bacteria, as well as the promotion of a balanced microbiota. Thus, the role of microbiota is resulting in novel and fascinating scenarios in regenerative medicine and surgery with unexpected progress.

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