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Review Article

Microbiology Section

Human Microbiome Engineering: The Future and Beyond

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ABSTRACT

Microbial flora of skin and mucosal surface are vital component of human biology. Current research indicates that this microbial constellation, rather than being inert commensals, has greater implications in health and disease. They play essential role in metabolism, immunity, inflammation, neuro-endocrine regulation and even moderate host response to cancer. Genetic engineering was a major breakthrough in medical research in 1970's and it opened up newer dimensions in vaccinology, large-scale synthesis of bio-molecule and drug development. Engineering human microbiome is a novel concept. Recombinant DNA technology can be employed to modify the genome of critical components of resident microflora to achieve unprecedented goals.

Keywords: Genetic engineering, Microbiome engineering, Microbial flora

INTRODUCTION

The adaptation of microbial flora on cutaneous and mucosal surfaces is a result of co-evolution over millions of years. This includes viruses, prokaryotes (bacteria) and eukaryotes (fungi and protozoa) [1]. They account for about 100 trillion microbial cells which even out number the cells of human host [2]. Soon after birth, environmental microbes establish colonization on skin, respiratory and intestinal tract. With exception of few symbiotic intestinal bacteria, majority of this microbiota is traditionally considered as commensals. In the light of current research, it is now evident that microorganisms which co-exist with human hosts, have essential role in maintaining normal physiology [3,4]. Microbial products and host-microbiome interactions are found to regulate metabolic activity, immune response, neuro-psychiatric functions and tumour macroenvironment [5-7]. Hence, introduction and maintenance of beneficial microbiome is likely to achieve a state of homeostasis decreasing the risk of infection, cancer and several metabolic and immune disorders. This is especially critical in view of emerging antimicrobial resistance among several medically important pathogenic bacteria. The scope of genetic engineering allows desirable alteration in microbial genome using recombinant DNA technology. Hence, microbiome research can be effectively enriched by genetic engineering [8]. Although microbiome engineering is still a concept, it has generated immense interest on its development and use. In this review, an attempt was made to describe the potential applications, challenges and limitations of microbiome engineering in relation to human health.

DEFINING MICROBIOME

The traditional microbiological approach to infective diseases was mainly focused on demonstrating pathogenic organisms by culture-based methods, establishing its pathogenic role as per Koch's postulates. However, it precludes the study of vast majority of commensals and pathobionts, which also determine the pathogenic potential of the causative agents. The term microbiome indicates the collective genomes of the microorganisms residing inside and on the human host [3]. Genomes of microbial communities in these ecological niches are considered as an extension of human genome. The microbial metagenome complements human genome by providing several bioactive molecules and enzymes which are imperative in digestion, metabolism and immune regulation [4].

Human Microbiome Project was the first systematic approach to study human microbial consortia [9]. Its main objectives were to develop a reference set of genome sequences of human microbiome and to investigate association of altered microbiome in various diseased conditions. Since majority of these microbes belong to unexplored and undescribed strata with unknown growth requirements, culture-dependent methods are invariably futile for a comprehensive study. The use of 16S rRNA sequencing, in conjunction with advanced techniques like shot gun meta-genome sequencing, proteomic, metabolomic and transcriptome analyses, paved the path of effectively understanding the inter-relationship of infinite culturable and non-culturable microbial species with human host [10,11].

IMPLICATIONS IN HEALTH AND DISEASE

Innate and acquired immunity are the two elemental immune mechanisms of immune system [12]. In the light of recent researches, it became evident that immune defence of human body is not entirely dependent on its own cells and molecules. The colonizing microbial cells, both in planktonic and biofilm forms, interact among themselves and the host immune system by establishing interlinked robust networks of nutrients, metabolites and bio-active molecules, contributing to colonization resistance and moderation of immune response [4,13]. It complements hosts nutrition by providing vitamins and carbohydrate metabolizing enzymes [4]. Colonization resistance is related to the inhibitory effect of well adapted intestinal microflora which effectively limits the nutrient resources available to the invading pathogens. Given the enormous number and diversity of microbial species, the interactions between host immune system and microbiome are not fully recognized. However, these interactions are mutually beneficial. In case of vaginal mucosa, the lactic acid and hydrogen peroxide produced by vaginal lactobacilli are critical in reducing the risk of vaginosis [14]. The development of a protective mucus layer derived from goblet cells depends on nature and density of local microbiota [4]. Blood group antigens in outer mucus layer of gut were found to favour the proliferation and retention of *Bifidobacterium* and *Bacteroides* spp. in secretor individuals [2]. These organisms produce essential nutrients for colonic epithelia (e.g. proprionate and butyrate) and antimicrobial products inhibiting other microbes. Altered microbiome has been found more frequently in patients with infection, autoimmune diseases, allergies and cancer in comparison to healthy individuals, indicating its possible influence on immune system [15-17]. The evidence based implications of these associations are listed in [Table/Fig-1].

Attributes of microbiome	Therapeutic implications
Compete with pathogens for nutrients	Colonization resistance, prevention or treatment of infections
Bacteriocin and other inhibitory products	
Biofilm formation	
Alteration of pH (vagina)	
Supplement nutrition and metabolism	Obesity, malnutrition, diabetes and dyslipidemia
Regulate immune response	Hypersensitivity and autoimmune disorders
Educate Immune system	Prevention of immune disorders and cancers
Anti-inflammatory products	Inflammatory Bowel Disease
Altered microbiota	Diagnostic and prognostic marker
Undescribed microbe / microbial genome	Source of novel bio-molecules Diagnostic and prognostic marker Evolutionary marker
Modulate cancer macroenvironment	Prevention of malignancies
Nuro-endocrine factors	Prevention or treatment of anxiety, depression, psychiatric or cognitive disorders

[Table/Fig-1]: Implications of microbiome

The greatest diversity and density of microbial consortia is found in large intestine owing to its nutrient-dense and stable ecosystem [18]. Consequently, it has been studied more extensively in comparison to cutaneous and other mucosal microflora. Evidences from microbiome studies in human as well as animal models suggest a strong association of gut microbiota with metabolism.

Faecal microflora transplantation (FMT) or stool transplantation has been utilized as a therapeutic modality in various gastrointestinal disorders. It has also been used to investigate the metabolic and health effects of intestinal microflora in various research models. It refers to the process of transplantation of colonic microflora from a healthy donor to a recipient. The faecal infusion can be administered in the form of enema or through orogastric tube. Capsules with freeze dried faecal microbiota can also be given orally [19]. There is evidence that the nature of colonic microflora has crucial role in nutrition. Malawian children suffering from severe acute malnutrition were found to harbour altered microbiota which induced weight loss, nutritional deficiency and other features of malnutrition in gnotobiotic mice on faecal transplantation [20]. Conversely, faecal transplant from obese individuals resulted in rapid weight gain in germ-free mice. The advantages of promoting the growth of innocuous intestinal flora had already been utilized for therapy to replace pathogens and establish eubiosis.

Probiotics can be defined as “a preparation of oral product containing viable, defined microorganisms in sufficient numbers, which alter the microflora in a compartment of the host and by that exert beneficial health effects in this host” [21]. Various probiotic preparations such as capsule, tablets, suspensions and freeze-dried powder, granules and pellets are available commercially. These constitute of a variety of beneficial intestinal bacteria (e.g. *Lactobacilli* and *Bifidobacteria*) and yeasts (e.g. *Saccharomyces*) which on ingestion beneficially influences the nutrition of the host. In contrast, prebiotics donot include any microbial agents both in viable or non-nonviable form [21]. Rather, it refers to non-digestible food ingredients which stimulate the activities and/or proliferation rate of normal intestinal commensals. Fructo–oligosaccharides, soybean oligosaccharides, galacto-oligosaccharides, transgalactosylated oligosaccharides and inulin are common examples of prebiotics [22]. Unlike dietary fibres, these stimulate one or a limited number of colon bacteria selectively. In addition, these also facilitate calcium absorption, increase fecal weight, shorten intestinal transit time and decrease lipid levels in blood [21]. Patients with diseased conditions associated with intestinal dysbiosis, i.e. irritable bowel syndrome, ulcerative colitis, Crohn enteritis, necrotizing enterocolitis and *Clostridium difficile* infection as well as metabolic disorders like obesity, dyslipidemia and diabetes mellitus, are found to respond adequately to probiotics, prebiotics and faecal transplantation [21]. These therapeutic modalities alter the intestinal microbiota producing beneficial health effects in the host. Identification of the vital constituents of healthy microbiota and their products are likely to widen the scope of bacteriotherapy. Furthermore, the fast emerging resistance to existing drugs has generated immense interest on the development of novel antimicrobials. Microbiome research aims at generating database of functional genome maps of commensal microbial communities which till date remained largely unexplored. This increases the possibilities of identifying newer antibiotics and bio-active molecules [7].

NEWER DIMENSIONS OF MICROBIOME

Regional, temporal and interpersonal variations are characteristic features of human microbiome [1]. Depending on age-groups, dietary habits and geographical locations, considerable interpersonal variations in metagenomics have been observed. Microbial communities adapted to the acidic, fatty acid and salt- coated inhospitable skin surfaces are distinct from that of anaerobic, nutrient-rich, bilious environment of intestine [1,2]. Even in the same body sites of an individual, the microbiome density and diversity may change over a period of time. The initial and most important source of human microbiome is maternal vaginal microbiota which is transferred to newborn during the passage though birth canal [2,23]. Secretory IgA of breast milk protects the infant from external microbial infections and also directs the maturation of immune system complying with the microbiota derived from the mother. Pertaining to the weak complement fixation ability of secretory IgA, response of innate immune system to microbial antigens bound to IgA is mostly tolerogenic [2]. Several studies have supported the fact that maternal microbiota and breast milk have decisive role in determining the colonizing flora in infant, at least in the initial months of life [2,23]. Infants delivered by Caesarean-section were found to have greater risk of developing asthma and other allergic illnesses in later life [2]. It also indicates the possible role of microbiome in educating human immune system.

Continuous alteration of microbiota due to indiscriminate drug use, adaptability for current lifestyle has health implications. With the increase in sanitation and antibiotic usage, the occurrence intestinal infections have dropped drastically with a proportionate increase in autoimmune, inflammatory, metabolic and malignant diseases. The deleterious effect of chronic antibiotic therapy on colonic microflora is a growing health concern. The occurrence of *Clostridium difficile* associated disease has increased significantly over previous decades in both hospital and in community [19]. The hygienic, nutrient rich, low-fibre food and random use of antibiotic may have a major role for causing a society-wide shift in gut microbiota [18]. Consequently, the depletion of large varieties of both symbiotic and pathogenic bacteria that favourably attune the immune system rendered it prone to immune over reactions, metabolic disorders and cancers [4]. Owing to its anti-inflammatory properties, Tregs were initially assumed to promote tumour progression by suppressing inflammation [24]. Contrary to this impression, Tregs induced by prior exposure of gut microbiota in conjunction with IL-10 has been found to impart systemic anti-tumour effects in animals [5]. Microbiota-host interaction results in anti-inflammatory tolerogenic response in gut mediated by Tregs and anti-neoplastic effect without adverse inflammatory tissue damage systemically associated with up-regulation of hormones, IFN-γ and IL-10 [6].

MICROBIOME ENGINEERING

Currently, the focus of application of microbiome knowledge is mainly on treatment and prevention of dysbiosis and associated conditions. However, it has not been utilized to its full potential due to lack of complete understanding. A large fraction of human microbiota is yet to be identified. However, the discovery of dominant or unique members of microbial communities serving specific beneficial functions is likely to bridge this gap and might become the mainstay of bacteriotherapy for several conditions in future [25]. Microbiome engineering will widen this scope to suit preventive, therapeutic and diagnostic needs.

The observation that gut microflora of peoples in rural tribal areas of Africa (Hadza of Tanzania) and South America (Yanomami of Venezuela and Matsés of Peru), significantly differs from that of North American citizens in their fermentative properties for plant fibres, has led to proposition that tribal population is protected from metabolic, immune disorders and cancers because of their unique microbiota in gut [13]. It is possible that primitive man had greater diversity of ancestral microbial communities which were imperative for digestion of unprocessed dietary fibres and protection against wider spectrum of pathogenic microbes [26]. Given the fact that replacing the ancestral gut microbiota in total may not be feasible, identification and supplementation of beneficial microbe may be an easier choice. Since, not much is known about the ancestral microbial communities, their adaptability in modern human intestine without adverse immune reaction is unlikely. However, their essential traits could be incorporated in existing inert gut commensals using genetic engineering to achieve the same result. Microbes can be engineered to ferment complex sugars and fibres, stimulate mucus secretion, and produce short-chain fatty acids and butyrate calibrating immune system by inducing Tregs [4,27]. Likewise, modification of mucosal or skin microflora to prevent infections requires incorporation of bacteriocin, antimicrobial peptide and biofilm genes into microbial genome of dominant commensals. Besides dietary modulation, microbes may be programmed to stimulate beneficial Tregs to impart anti-neoplastic effect in the host [6].

New evidences from animal studies suggest there is a strong link between gut microbiota and brain functioning. It is observed that the abnormal hormonal output in response to stress in germ-free mice normalizes drastically when implanted with gut microbiota [7]. Social behaviour and personality of donor mice can be induced in germ-free mice through transfer of gut microbes. Alteration of gut flora in mice resulted in abnormal behaviour that is comparable to depression, anxiety and autism in human [7]. It is postulated that microbial metabolites which are neuroactive in nature influence the brain. Current research is focused on discovering and engineering these novel molecules, often described as 'psychobiotics' [28].

FUTURE PROSPECTS OF MICROBIOME ENGINEERING

Given the rapid growth of medical science and technology, it is not impractical to think of widening the applications of microbiome research beyond its realm. Microbial interactions with host and other microbes are based on its ability of sensing environmental signals. There is immense interest on optimizing a DNA memory device which would enable us to reprogram these microbial interactions [8]. A particular ecological niche can be populated by engineered microorganisms which release specific molecules (e.g. antibiotics, anti-inflammatory molecules, cytotoxins, growth factors and protective antigens) in response to suitable stimuli (e.g. infection, inflammation, neoplastic change, tissue damage, etc) specifically in a contained area [27]. At the least, microbes can also be programmed to secrete different signal molecules which can serve as biomarkers for specific disease. Studies on animal models and in vitro experiments have shown encouraging results of microbiome engineering which are listed in [Table/Fig-2] [29-41].

Targeted drug delivery is especially important for vaccinology for effective uptake of the vaccine. In future, commensals may express a variety of protective antigens on demand basis when the protective antibody level falls below a predetermined critical level [27]. The tissue tropism of microbes is due to their receptors expressed on human cells. Hence, there is possibility to develop innocuous resident bacteria which recognize tumour antigens as receptors and specifically kill or infect cancer cells [6]. The microbial antigens in conjunction with MHC-I molecules expressed on these cells will render them susceptible to cytotoxic T cells and natural killer cells.

Engineered commensal microbe	Induced change by genetic engineering	Result	Reference
Bifidobacterium longum	Expression of recombinant *Salmonella* flagellin on the cell surface	Protection against *Salmonella typhimurium* infection in mice	Yamamoto et al., (2010)[29]
Lactobacillus casei	Expressing protein C of pneumococci	Reduction of nasopharyngeal colonization of pneumococci in mice	Hernani et al., (2011) [30]
Lactococcus lactis	Express recombinant LcrV antigen of *Yersinia pseudotuberculosis*	Protection against Y. *pseudotuberculosis* by both oral and systemic route	Daniel et al., (2009) [31]
Lactococcus lactis	Express recombinant hemagglutinin of H5N1 influenza virus (vaccine)	Antibody response and immunity against H5N1 influenza virus	Lei et al.,(2010) [32]
Lactobacillus jenseni	Colonized vaginal mucosa and secretion of cyanovirin-N (HIV-1 entry inhibitor) locally in monkeys	Protection from simian HIV infection	Lagenaur et al., (2011) [33]
Caulobacter crescentus	Displaying anti-HIV antibodies on its surface-layer	Neutralization of HIV	Duval et al., (2011) [34]
L. jensenii	Expression of anti-HIV chemokine RANTES and C1C5 RANTES	Inhibition of HIV infection in CD4+ T cells and macrophages	Vangelista et al., (2010) [35]
E.coli	Production of AI-2 and CAI-1 (autoinducers) in high concentration	Quorum sensing mediated repression of cholera toxin production by *V. cholerae*.	Duan et al., (2010) [36]
E.coli	Expression of pyocin S5(bacteriocin) on detection of 3-oxo-HSL of *P.aeruginosa*	Inhibition of planktonic growth and biofilm formation of *Pseudomonas aeruginosa*	Saeidi et al., (2011) [37]
E.coli	Chimeric cell wall lipopolysaccharide mimicking GM 1 ganglioside	Neutralized cholera toxin and heat-labile enterotoxin of *E.coli*	Focareta et al., (2006) [38] Paton et al., (2005) [39]
Lactobacillus paracasei	Expression of recombinant Listeria adhesion protein	Competitive inhibition of *Listeria monocytogenes* to bind with its receptor	Koo et al., (2012) [40]
Lactococcus lactis	Expression of surface-associated flagellin of *Bacillus cereus* CH strain	Competitive inhibition of adhesion of intestinal pathogens like *Salmonella enterica*	Sanchez et al., (2011) [41]

[Table/Fig-2]: Successful outcomes of microbiome engineering

Liver helps in detoxification of large number of endogenous and exogenous metabolites and drugs. Furthermore, liver and kidney are the two main avenues of excretion of most drugs and chemicals. Impaired function of these organs results in accumulation of toxic products in body. Microbiome engineering may enable resident microbiota to supplement functions of liver and kidney by detoxifying and metabolizing harmful chemicals.

CHALLENGES IN MICROBIOME ENGINEERING

The prospect of microbiome engineering is limited by the lack of adequate knowledge about human microbiome and its interactions with host and environment. The approach to study microbiome has been shifted from simple sequencing to the systematic analysis of microbial metagenome, proteome, metabolome and transcriptome. It has generated enormous volume of data which is difficult to organize, analyse and reproduce [11]. Newer infrastructures, softwares and models are in development to address this issue [10]. The impact of microbiome engineering on human health will be radical. It is more likely to affect other aspects of life, i.e. life span, food habits and even social behaviours. This may raise the question about its safety. The over-dependence on microbiome for defence against invading pathogens, toxic antigens and cancers, will deteriorate the functional integrity of own immune system over a period of time. Moreover, spontaneous mutations are inevitable attributes of any living cell. It often leads to carcinogenesis, microbial drug resistance and exaltation to virulent prototype. Hence, there is possibility that beneficial microbes may mutate and turn up as pathogenic or carcinogenic. This requires a mechanism to eliminate engineered microorganisms if necessary before initiating widespread use of microbiome engineering [27].

CONCLUSION

The introduction of microbiome concept has revolutionized the insight into health and disease. Pathogenic basis of several immune disorders and familial diseases are now adequately explained by the diversity of microbial communities in host. Microbiome engineering has the promising prospect to improve human health as it enables us to step forward and manipulate microbiota in a variety of ways. However, its benefits have to be balanced against the risks that may incur from its in judicious uses.

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