Second Skin ™ microbe patches: Restoring the symbiosis of the skin microbiome in Atopic Dermatitis patients through microbe transplantation

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ABSTRACT

Atopic dermatitis (AD) is an inflammatory skin disease, with severe impact on the lives of patients suffering from it. Recent studies show that its pathogenesis involves activity of microbes and dysbiosis of the skin microbiome occurs, with *Staphylococcus aureus*, a gram-positive member of the Firmicute phylum, being dominantly present, potentially causing further infections. Successful treatment usually involves administration of antibiotics, with the known negative side effects on other, commensal microbiota in the body. The goal of this project is the development of a patch that contains healthy donor skin-microbiota to restore symbiosis of the skin microbiome of AD patients, offering a healthier alternative for treatment.

PROBLEM DEFINITION AND AIM

The skin is the largest organ in the human body and plays an important role in the defense against toxic agents and infectious organisms. *In utero* the skin is sterile, but analyses of neonate skin after birth indicates that babies delivered via vaginal birth have a skin microbiota similar to the vaginal microbiota of their mother and babies that were delivered by Caeserion section had skin microbiota similar to their mother's skin microbiota (Grice, 2014). How long these differences exist is not entirely clear, since vaginal bacteria are not usually found in a later stage on the skin; The skin is an dynamic environment and additional factors can contribute to the diversity of its microbiota, like lifestyle-choices, genetic predisposition, gender, geography and ethnicity.

It is estimated that over 100 distinct species making up a total of 1 million microorganisms colonize each square centimeter of our skin (Zeeuwen, Kleerebezem, Timmerman, & Schalkwijk, 2013). Generally, the four dominant phyla of bacteria residing on the skin are the Actinobacteria, Proteobacteria, Firmicutes and Bacteroidetes. Like in other sites of the human microbiome, variability between individuals and temporal variability within the same person is high (Grice, 2014).

Maintaining a healthy skin microbiota is beneficial, since it controls colonization by potentially pathogenic microorganisms and is thereby necessary for optimal skin immune fitness (Naik et al., 2012). Besides the symbiotic relationship that these microbes share with skin tissue they live on, they also seem to partly influence the course of associated disease. Various dermatological disorders are being linked to changes in the structure of the skin microbiome (Ganju et al., 2016).

Atopic dermatitis (AD), commonly referred to as eczema, is a chronic, relapsing, and often intensely pruritic inflammatory disorder of the skin. The effects of the disease on the quality of life cannot be underestimated; AD patients suffer from itching, fatigue, sleep deprivation, activity restriction and depression (Tollefson & Bruckner, 2014). Evidence of recent studies point to microbial involvement in the pathogenesis of AD. Microbiome analyses through 16S rRNA gene sequencing have revealed temporal dysbiosis of the skin microbiome, dominated by *Staphylococcus aureus* during AD flares (Kong et al., 2012). Many patients with AD have sudden exacerbations of their disease that can be attributed to an active infection with *S.aureus.* Clinical signs of such a bacterial infection are pustules, oozing and honey-colored crusts, and less commonly fever and cellulitis (Tollefson & Bruckner, 2014). Often intensified antibiotic cocktails might be necessary to target skin-colonizing *S.aureus* (Kobayashi et al., 2015). But the use of antibiotics over a prolonged period of time is not ideal, since it can have undesirable effects on the commensal microbiota in the gut and other distal sites.

The aim of this project is to develop the Second Skin ™microbe patches that could provide a more healthy and alternative treatment for people suffering from AD by restoring their skin microbiota through re-colonization of commensal bacteria that are common on healthy skin tissue.

APPROACH

Similar to the approach of van Nood et al, which utilized fecal transplantation therapies to treat *Chlostridium difficile* infections (van Nood et al., 2013), the Second Skin ™ microbe patches will also make use of transplantation, but in this case to re-establish a commensal and healthy skin microbiome.

As mentioned before, different factors contribute to the diversity of the skin microbiome. With this fact in mind, samples of the skin microbiota of healthy, volunteering individuals of different gender and ethnicity will be taken and analyzed by 16S rRNA sequencing or Maldi-tof MS. The sequence files will be compared to the results of other studies to make sure that the samples sufficiently match the skin microbiota of the targeted patients. The samples will be grown under appropriate conditions until a density is reached that makes transplantation to the actual patch possible. Having different skin microbiota samples according to ethnicity and gender will in the end lead to the production of a wide range of Second Skin ™ microbe products, according to the patients needs.

The prototype of the Second Skin™ microbe patch will consist of a membrane that forms the lower part of the patch that makes contact with the damaged skin and to which the commensal bacteria will be applied. This membrane is coated with liquid medium, containing nutrients that will enable the bacteria to thrive. Additional components will be added to the medium to adjust the pH, guaranteeing an ideal environment for growth. The membrane will contain micropores that are big enough to ensure oxygen flow to the bacteria-containing

membrane, but also small enough to block microbes from the outside air, that possibly could invade the membrane and contaminate the commensal bacteria culture.

The top op the patch will have an additional layer that is bigger than the membrane itself, completely covering it. This layer will have a thin stretch of strong adhesive material around its edges to safely secure the patch onto the skin. Just like the membrane below it, the top layer will serve as a micropore filter, passing oxygen to the membrane. This layer should be made of material strong enough to prevent tearing of the patch after exposure to moderate physical stress.
Compared to crème as a medium that could be applied, a sturdy patch has the advantage that it does not smear or wash of easily, so patients can follow their daily routine, without having to pay extra attention or care.

The whole manufacturing and assembly process will be occurring under sterile conditions. The Second Skin ™ microbe patches will be available in different sizes, to cover a broad spectrum of damaged skin tissue.

APPLICATION AND RESULTS

The first clinical trials will be held with a cohort of volunteers suffering from AD, divided into groups of gender and ethnicity. The trials will be supervised and executed by qualified medical doctors and pediatricians. The Second Skin ™ microbe patches suited for the patients accordingly, will be applied onto the damaged skin tissue. The skin microbiota of the patients will be analyzed at different time intervals to observe direct results. The used patches will also be analyzed to determine the depletion time of the commensal microbiota on the membrane patch.

It is expected that the transplanted commensal bacteria of the Second Skin ™ microbe patches will ameliorate *S.aureus* colonization of the damaged skin, thereby reducing the symptoms of AD.

Additional trials could be held, where the Second Skin ™ microbe patch is combined with gastrointernal probiotics for additional effect, since studies suggest, that the use such probiotics also reduces the severity of AD (Foolad, Brezinski, Chase, & Armstrong, 2013).

After a successful trial period and if production of the Second Skin ™ microbe patch is approved by the respective authorities, the production line could be further expanded. Researchers have indicated that family members are inclined to share microbiota with each other (Song et al., 2013). This would pave the way to personalize and thereby hopefully improve treatment of AD by producing tailor-made patches that contain commensal skin microbiome samples of family members of the respective patients.

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