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Extended Abstract
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Wound Care and Epidemiology 2019: Broad spectrum repairing properties of an extract of Aquaphilus dolomiae on in vitro and ex vivo models of injured skin - Noizet Maite, P. Bianchi, MF Galliano, A. Caruana, S. Bessou-Touya & H. Duplan and J. Brandner - University Hospital Hamburg, Germany

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Several studies have shown the clinical efficacy of Avne thermal spring water (TSW) in atopic dermatitis, psoriasis or in reducing erythema post laser resurfacing. In addition to these soothing and immunomodulatory properties, in-vitro experiments have also demonstrated effects of Avne TSW on stimulation of keratinocyte differentiation and improvement of membrane fluidity, suggesting a potential effect on skin barrier and repair. An investigation of the deep aquifer of the Avne TSW pointed out a new microorganism as a potential source of these unique properties. Based on its distinctive phenotypic and genotypic characteristics, this newly identified strain was assigned to a new genus, as a representative of a novel species called Aquaphilus dolomiae. It is a chemoorganotrophic non-spore-forming bacterium of the b-Proteobacteria class. In the present study, the activity of AD-S0, an original biological extract of A. dolomiae, was evaluated on invitro models of injured skin. The compound showed positive properties on primary fibroblast proliferation and keratinocyte migration. When formulated, it favored skin re-epithelialization on a 3D model of wounded skin explants. Moreover, we showed that AD-S0 could prevent wound infection by up-regulating numerous antimicrobial peptide genes and inducing hBD2 peptide release. All together, these results show broad repairing properties of the A. dolomiae extract S0, helping skin repair and preventing complicated wounds.

Previous experiments by our group have indicated the regenerative effects of a spring water (Comano), which was possibly associated with the native non-pathogenic bacterial flora. The present study aimed to confirm these regenerative properties in a human ex vivo experimental model in the context of physiological wound healing. Human 6-mm punch skin biopsies harvested during plastic surgery sessions were injured in their central portion to induce skin loss and were cultured in either conventional medium (controls) or medium powder reconstituted with filtered Comano spring water (treated samples). At 24, 48 and 72 h the specimens were observed following staining with hematoxylin and eosin, Picrosirius Red, orcein and anti-proliferating cell nuclear antigen. Compared with the controls, the treated samples exhibited reduced overall cell infiltration, evidence of fibroblasts, stimulation of cell proliferation and collagen and elastic fiber regeneration. In the spring water, in addition to 12 resident non-pathogenic bacterial strains exhibiting favorable metabolic activities, more unknown non-pathogenic species are being identified by genomic analysis. In the present study, the efficacy of this ‘germ-free’, filtered spring water in wound regeneration was indicated. Thus, the Comano spring water microbiota should be acknowledged for its regenerative properties.

The role of microbiota in controlling the balance between health and disease is a current topic of study due to its potential to be used for novel therapeutic approaches. In particular, the local application of bacteria to enhance wound healing has previously been reported. These favorable effects may be related, though not exclusively, to certain anti-inflammatory and antibiotic actions of bacteria. Previous experiments by our group have indicated that the Italian calcium magnesium bicarbonate-based Comano spring water (Comano, Italy) improves skin regeneration: In an in vivo rabbit wound model, it was identified that the topical administration of the Comano spring water increased keratinocyte proliferation and migration, and favorably modulated the regeneration of dermal collagen and elastic fibers; while in in vitro cultures of human skin fibroblasts, it was observed that cells maintained in conventional Dulbecco's modified Eagle's medium (DMEM) with

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20% Comano spring water exhibited a 31% higher vitality than control cells maintained in conventional DMEM alone after 72 h. Other studies have demonstrated the anti-inflammatory effect of the spring waters of Avène and La Roche-Posay in France, effective in activating toll-like receptors due to the specific actions of the non-pathogenic bacteria Aquaphilus dolomiae and Vitreoscilla filiformis, respectively. The Comano spring water also exhibits a diverse non-pathogenic bacterial flora. To further investigate the regenerative effects of the native bacterial flora of the Comano spring water, the present study evaluated the efficacy of spring water treatment in a human ex vivo model of physiological wound healing. The study was performed at the Plastic Surgery Unit in the Department of Clinical-Surgical, Diagnostic and Pediatric Sciences, in cooperation with the Histology and Embryology Unit in the Department of Public Health, Experimental and Forensic Medicine at the University of Pavia (Pavia, Italy). The results of the current research may aid the development of novel clinical approaches for tissue regeneration and wound healing within the modern concept of 'natural' medicine. Additionally, they may provide novel scientific data on thermalism and indicate its therapeutic use with a rational basis.

Spring water collection and processing Comano spring water was collected in January 2014 from the Comano spring by an aseptic procedure. Briefly, a single operator wearing sterile surgical gloves collected 3,000 ml water with a sterile 60-ml syringe. The samples were poured into three sterile 1-L containers, stored at 4°C and transported to the Histology and Embryology Unit of the University of Pavia (Pavia, Italy). After 2 days, the spring water was filtered through 0.20-µm pore cellulose nitrate membranes (Nalgene 0.2 Analytical Filter Units; Thermo Fisher Scientific, Inc., Waltham, MA, USA), stored at 4°C in sterile 100 ml ampoules and used as described below. All ex vivo experiments were performed within 3 months of water filtration and storage. Human skin samples were obtained from anatomical specimens harvested during sessions of elective abdominoplasty or reduction mammoplasty performed on 6 healthy female patients (age range, 43–56 years). The specimens were sampled by a surgeon in ~6×6-cm segments and conserved in sterile containers filled with saline solution enriched with 1% (10,000 U/ml) penicillin (Biowest, Nuaillé, France), then transported to a partner laboratory for further processing. The time delay between tissue harvesting and the initiation of laboratory procedures was ~45 min. The study was conducted in accordance with the 1975 Declaration of Helsinki, informed consent was obtained from all patients and the protocol was approved by the Ethics Committee of Salvatore Maugeri Research and Care Institute, Pavia, Italy.

The skin samples were sufficient for the harvesting of 6 mm-punch biopsies that in turn were injured in their central portion with a sterile 3-mm circular punch to establish skin loss in each sample as described previously (10–12). The injured specimens were placed into Transwell inserts for 24-well multiwell plates (membrane pore size, 0.40 µm; Constar insert, 0.33 cm2; Corning Incorporated, Corning, NY, USA). Each of the 6 skin specimens was used to harvest paired control and experimental samples. The control samples were cultured in DMEM with 1.0 g/l D-glucose (Biochrom, Ltd., Cambridge, UK), 10% fetal bovine serum (FBS), 1% penicillin (10,000 U/ml) and streptomycin (10 mg/ml), 1% gentamicin (all from Biowest) and 10 ml/l 200 mM L-glutamine (Eurobio Laboratoires, Les Ulis, France), and the central skin loss region was treated with a constant volume (200 µl) of sterile saline solution. The samples treated with Comano spring water (treated samples) were cultured with DMEM powder without NaHCO3 with 1.0 g/l D-glucose (Biochrom, Ltd.) and 10 ml/l 200 mM L-glutamine, reconstituted with filtered Comano spring water and enriched with 10% FBS, 1% penicillin (10,000 U/ml) and streptomycin (10 mg/ml) and 1% gentamicin (all from Biowest). The central skin loss region was treated with a constant volume (200 µl) of filtered Comano spring water. Following treatment, the control and treated samples were incubated at 37°C for 24, 48 and 72 h.

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