FUCOSAN

Result Report
Pilot developments in medicine and cosmetics

Interreg
Deutschland - Danmark

EUROPEAN UNION

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This result report presents a compilation on the key findings provided by the partners working in the work package:

WORK PACKAGE 1 — Project management

WORK PACKAGE 2 — Project communication & PR

WORK PACKAGE 3 — Algae sources, cultivation and collection

WORK PACKAGE 4 — Fucoidan characterisation and database development

WORK PACKAGE 5 — Pilot developments in medicine and cosmetics

WORK PACKAGE 6 — Organisation and business models

The FucoSan project

Algae from the North and Baltic Sea serve as an important but yet under-exploited marine bio resource. Brown algae contain fucoidan - a polysaccharide with highly health-promoting activities that could be used in medicine and cosmetics. Fucoidans are also valued for their positive influence on inflammation, vascular supply and tissue regeneration.
With their antimicrobial properties, infections in the bone could potentially be treated. However, fucoidan varies in structure, composition and modifications such as degree of sulfation or molecular weight - depending on the origin and other factors. This leads to different, sometimes even opposing effects.

The FucoSan project aimed at generating systematic knowledge of fucoidans and their modes of action. In various test systems, the project partners investigated on the optimal fucoidan for each particular application. Over the last three years, the project established a network in the German-Danish cross-border region pooling the expertise of companies and research institutions. They are active in the fields of extraction and purification as well as in chemical and biological characterisation of fucoidans.

March 2017 – August 2020

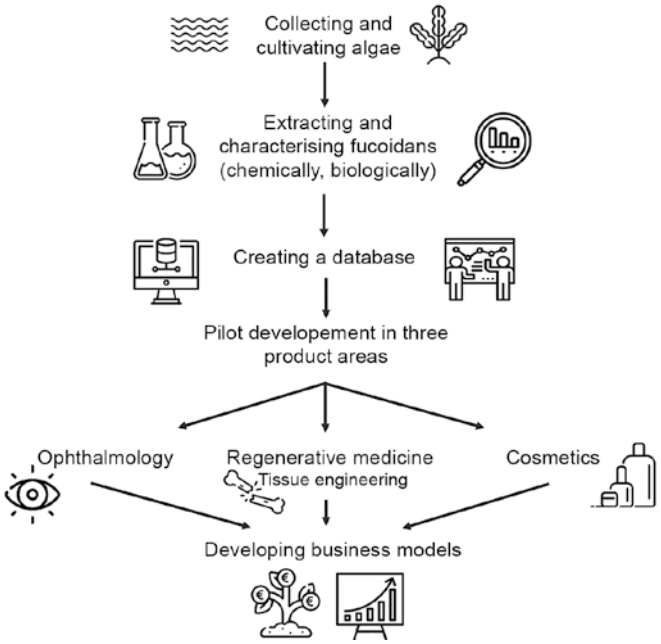
3.8 million Euros budget,
thereof 2.2 million Euros funds

8 partner organisations from
Denmark and Germany

Project aims

- ✔ Development of economically and ecologically sustainable processes to obtain brown algae from the Baltic Sea
- ✔ Setup of a database for the identification of suitable fucoidans
- ✔ Pilots for fucoidan-based applications in ophthalmology, regenerative medicine (tissue engineering) and cosmetics
- ✔ Establishment of a German-Danish value chain around the use of fucoidans

The FucoSan process chain



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Pilot development for medical and cosmetic fucoidan application

The transfer of scientific findings into value-adding processes is currently one of the greatest challenges in marine biotechnology. In the FucoSan project, an innovation network of researchers and interested companies was established exploring the developmental potential of the marine active ingredient fucoidan. Intense cooperation and experience exchange throughout the project's lifetime brought forward the idea of a new value chain. Data gained in frame of the pilot cases provide interested users with essential information on three possible application fields and thus enable them to enter into a (future) commercial use. As foundation for a later business development based on specific fucoidans and their chemical properties and biological activities, the project partners strove for application-oriented scientific findings in medical and cosmetic fields. Since fucoidan properties may vary, the most promising extracts were identified and the specific fucoidans were tested further

- for treatment of the eye-disease age-related macular degeneration (AMD, ophthalmology),
- in-vitro and in-vivo in the field of regenerative medicine / tissue engineering as well as
- for cosmetic use.

The additional data gained have been published in numerous scientific articles and conference presentations, and are being well perceived by the algae-related research community in the German-Danish cross-border region and all over the world as the echo on the project conferences in 2019 and 2020 confirmed.

Like the other scientific findings from the project, those from the three pilots are also available for scientists and companies interested in further development of fucoidan application possibilities in a preclinical and/or clinical environment. These results pave the way for development and possible launch of fucoidan-based medical products. For the cosmetic application, the pilot resulted in defined product specifications and the development of a prototype for a future skin-care product, which is close to market release by the end of the FucoSan project.
All these new, innovative approaches in fucoidan research open up completely new development opportunities for marine biotechnology especially in the German-Danish programme region and lay the foundation for the commercial use of this promising marine resource.

Ophthalmology
Age-related macular degeneration (AMD)

Regenerative medicine / tissue engineering for bone healing

Cosmetic

Pilot "Ophthalmology: Introduction of the challenge addressed

Age-related macular degeneration (AMD) is the major cause of severe vision loss in the elderly in the industrialised world. As the population ages, this problem becomes even more severe. The disease affects the patient's central vision - that is the ability to recognise faces, drive a car or read a book. Impaired central vision has a deep impact on the independence and self-determination of the affected person. To lose vision after a lifetime of independence, no longer being able to see the faces of families, to travel or to enjoy any other activity connected to fair vision can be very traumatic for a patient and relatives, and is a social-economic challenge for society[1].

The disease has many faces. Early forms show few symptoms for the patient. It usually takes an ophthalmologist to realise that the patient has a problem. These early forms, however, can progress in two different directions: the late dry and the late exudative form. The late dry form involves the slow degeneration of retinal tissue with an equally slow loss of vision, resulting in large areas of degeneration, called geographic atrophy. In the exudative form, abnormal vessels grow into the retina, causing oedema and a rapid loss of sight. Unfortunately, today we have only treatment options of the exudative form of the disease, but not for the early or dry form. Furthermore, the treatment for the exudative form may halt the disease of a while, however, in long term, vision loss cannot be prevented[2].

AMD is a complicated disease with many factors contributing to its development. Genetics may predispose people to a higher risk for developing AMD. Here, especially the complement system, a pathway originally developed to protect the body from danger, seems to be involved. Lifestyle is also very important with smoking being a contributing factor, and a healthy diet and exercise being protective. Furthermore, on the level of the retina, pathogenic factors include oxidative stress, inflammation and the secretion of pro-angiogenic cytokines. These contributing factors act already at the early forms of the disease and accumulate during time leading to the devastating effects in the later disease forms[3].

The retina is a fragile tissue with little regenerative ability. Treatments that are given when tissue has already degenerated are at best able to hold the disease – lost tissue is generally not regained. Therefore, it is of vital importance to develop new therapeutics that target the disease at an early stage, preventing the progression to the late forms and, most importantly, vision loss. For this, a therapeutic that targets several pathogenic factors at once would be the therapeutic of choice. Treating early, stopping the pathogenic pathways before vision is lost would not need to regenerate the tissue - it would stop its degeneration in the first place.

Motivation why fucoidan

As described above, AMD is a multifactorial disease which pathogenic pathways include oxidative stress, inflammation, and pro-angiogenic cytokines. Fucoidan has been investigated for its potential beneficial health effects before, and many of the found properties of fucoidan may also be helpful in AMD prevention. Fucoidan has been shown to prevent oxidative stress either by scavenging (or "catching") it before it does any harm to the tissue, or by inducing the intrinsic oxidative stress protection pathway in the cells, rendering the cell less vulnerable to oxidative stress. Also, fucoidan may interfere with many aspects of inflammation. Fucoidan has been shown to inhibit complement activation or the activation of macrophages (which are cells of the innate immune system), reducing inflammatory activation and damage. Importantly, fucoidan interferes with pro-angiogenic cytokines and may prevent abnormal blood vessel formation. Finally, fucoidan seems to have a general positive impact on health, e.g. reducing blood pressure. Therefore, fucoidan seems a very interesting choice to be investigated in the light of AMD application. However, fucoidan has hardly been tested in an ophthalmological context, and effects on cellular and tissue levels are highly dependent on the cells and tissues being tested – not all organs react alike to the same bioactive substance[4].

Moreover, fucoidan is not a single, defined chemical compound, created in a laboratory. Fucoidan is a natural product and its molecular composition and molecular effects are highly dependent on external factors, such as the species from which it is harvested and the life circumstances of the plant. In addition,

fucoidan has to be extracted from the plant and the way how the fucoidan is won may have a great impact on its molecular properties. Concomitantly, the effects fucoidan has on the cells and tissues are highly dependent on its chemical characteristics, such as molecular weight or degree of sulfation, with converse effects being found for different fucoidans. Therefore, when investigating the potential of fucoidan as a new preventive treatment option, these factors need to be carefully investigated in order to find the most promising fucoidan for further development.

Flow chart

The development of new therapeutics includes several layers of investigation, usually starting with chemical and biological characterisation. The effects on living systems are generally first tested in cell culture models, as a prerogative before testing in the living organism can be considered. This pilot, mainly conducted in University Medical Center Schleswig-Holstein, Department of Ophthalmology, paves the way for further development by establishing the biological characteristics in cell culture models, using fucoidans extracted and chemically characterised by the partners from Kiel University (CAU), University of Southern Denmark (SDU) and Technical University of Denmark (DTU).

We use various cellular test models to determine the effects of different fucoidans in properties interesting in regard to AMD (Figure 1). A uveal melanoma cell line (OMM-1) serves as a model for cells of the eye sensitive to oxidative stress. Most importantly, we work with cell models of the so-called retinal pigment epithelium (RPE), a cell type that maintains the photoreceptors and is indispensable for keeping up vision. For this, we use the human cell line ARPE-19. All soluble fucoidan-containing extracts provided by our partners CAU, SDU and DTU are first tested in ARPE-19 and OMM-1 (basic characterisation). With the data from these initial tests, we choose promising extracts for further assays. In the basic characterisation we test for toxicity as well as protective effects against different oxidative stressors (H_2O_2, TBHP (tert-butyl hydroperoxide) or erastin). In addition, the influence on VEGF secretion is determined in ARPE-19.
Non-toxic extracts which were protective or inhibited VEGF are further tested in primary RPE cells prepared from pigs' eyes. These cells are not as easily available as the cell lines used before but are much closer to the situation in the human eye. Again, toxicity and influence on VEGF secretion is tested. VEGF inhibiting extracts are further tested for possible anti-inflammatory properties. For this, we induce a pro-inflammatory stimulus with LPS (lipopolysaccharide), simulating bacterial infection and determine the concentration of secreted pro-inflammatory cytokines (IL-6, IL-8, TNF-α). Extracts which reduce VEGF and show anti-inflammatory effects in primary cells are considered promising candidates for further development for potential AMD therapies and interesting for further testing. This testing includes the evaluation on effects on RPE function, gene-arrays in a real-time PCR system, where we can determine how the relative gene expression of AMD and inflammation relevant genes is changed, and tests in organ cultures. Here, we clamp RPE-choroidal tissue in rings and insert them into perfusion chambers to determine VEGF secretion. Due to the interaction of the tissues in the culture, this model is closer to the real conditions in the eye. These experiments pave the way for further ongoing research, which will possibly lead to clinical trials.

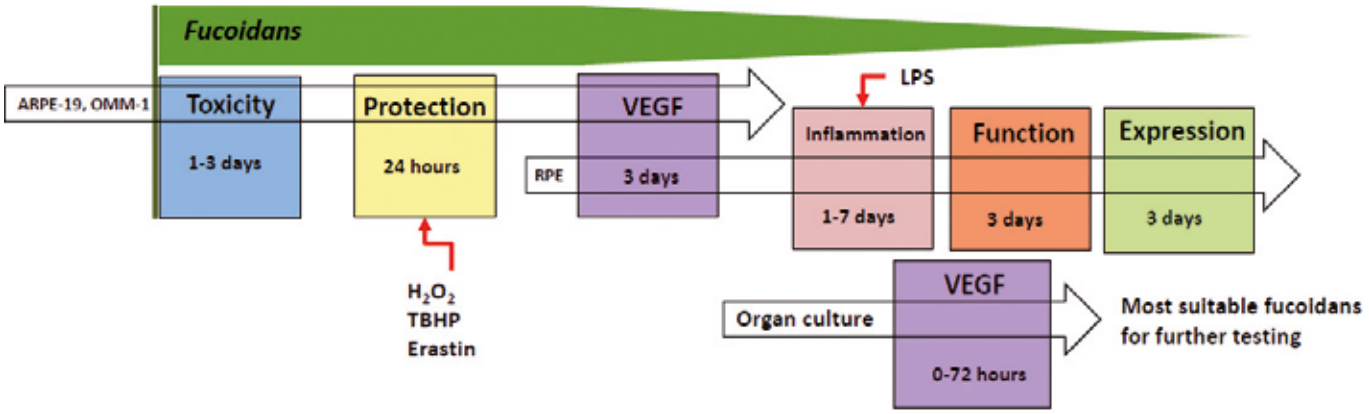

Figure 1: Overview about conducted bioassays and used model systems.

Main findings

All lot of heterogeneous data concerning the effects of fucoidans on ocular cells, were gathered. Each extract can affect the cells differently. We tested more than 70 fucoidans from eight different seaweed species concerning oxidative stress protection, toxicity and VEGF. In general, fucoidans did not reduce cell viability in the tested cell lines[5]. Fucoidans of *Fucus vesiculosus, Fucus serratus, Fucus distichus* subsp. *evanescens (FE), Laminaria digitata* and *Saccharina latissima (SL)* were anti-oxidative for OMM-1 and VEGF inhibiting in ARPE-19, showing all a high binding affinity to VEGF. Additionally, SL fucoidan was protective in ARPE-19 and lowered VEGF secretion in RPE cells[6]. The species of SL seems very promising concerning useful activities of fucoidans and were investigated further. On the other hand, crude fucoidan from FE inhibit VEGF slightly, was not protective and showed some negative effects for wound healing and phagocytosis of RPE[7]. The purity and fucose content is an important factor. We tested enzymatically treated SL extracts, which resulted in a high content of fucoidans, low content of alginates and a high degree of purity. And indeed, these SL extracts were the most effective extracts in terms of VEGF inhibition of ARPE-19 and RPE cells[8].

Also, fucoidans from *Laminaria hyperborea* (LH) seem very promising, because they showed antioxidative effects and lowered VEGF in ARPE-19 as well as RPE cells, with the effects depending significantly of the molecular weight of the fucoidan. The bigger the fucoidan, the more angiogenic and anti-oxidative it is9. The biggest LH fucoidan also increased the intracellular level of glutathione, which is an important anti-oxidative defence molecule in the cell. Moreover, it showed protective effects against erastin in OMM-1 and ARPE-19. Erastin induces iron dependent, oxidative cell death.

Taken together, we have identified the enzymatically treated SL fucoidan and the big LH fucoidan as the most promising candidates which are of high interest for further development. They are currently being further tested concerning effects on RPE function, in gene arrays, and in perfusion organ culture (Figure 2).

Figure 2: RPE/choroid rings in perfusion chambers. Media samples of a fucoidan treated and an untreated organ culture are taken after passing through the chamber, to compare the VEGF secretion.

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Pilot "Tissue Engineering”:
Introduction to the challenge addressed and motivation for choosing fucoidans

Fucoidans, sulphated polysaccharides extracted from brown seaweed, have raised interest in bone health applications due to their ability to modulate bone formation and associated vascularisation processes. Bone is a highly vascularised tissue. Accordingly, bone fracture healing as well as general bone tissue maintenance depend significantly on a functional vascular system. Thus, therapies to enhance bone formation and vascularisation are highly relevant for an aging population suffering from an increasing incidence of musculoskeletal diseases. Furthermore, in patients with systemic diseases such as diabetes or osteoporosis, bone health and vascularisation are either directly impaired by the underlying molecular mechanisms, or might be negatively affected by associated medical therapies. In contrast to the majority of musculoskeletal diseases which benefit from pro-angiogenic therapies, blocking of vascularisation is a widely-used therapeutic concept for bone tumour treatment. These examples demonstrate the central role of vascularisation processes in bone health and the therapeutic potential of bioactive compounds modulating the blood vessel formation in bone tissue.

Fucoidans are characterised by heparin-like properties and the ability to bind to vascular endothelial growth factor (VEGF). Thus, fucoidan might be used to control VEGF levels, and thereby also angiogenic processes, in bone tissues or bone tumours. It might also be used for the site-specific delivery of VEGF. Nevertheless, fucoidan extracts are natural products with a high variation in their content of biologically-active compounds, and thus need in-depth evaluation in terms of endotoxins, cytotoxicity and their detailed molecular biological impact on the individual cell types in the bone. As reported in the literature, pro- and anti-angiogenic effects of fucoidans might correlate with their physicochemical properties, such as the molecular weight or the degree of sulphation. In most cases, extracts from brown algae contain a series of biologically-active compounds and vary in their fucose content, so that the structure- and function-related mechanism characterising their biological activity remains elusive. The aims of this work package were to achieve a better understanding of whether fucoidans might be beneficial for bone health, to reveal the distinctive molecular functions, and to select the most promising extracts for the specific applications.

First, the cellular and molecular effects and the biological safety of fucoidans were investigated using in vitro models relevant for bone formation and vascularisation as a first screening system. The effects of fucoidans on bone formation and vascularisation were investigated and associated target molecules in human blood-derived endothelial cells and mesenchymal stem cells (MSC) in individual cell culture systems were identified. This was followed by functional assays using microtissue-like co-cultures consisting of both cell types.

The most effective fucoidan extracts selected are currently being further evaluated in small and large animal models. These in vivo animal studies are 1) to assess the efficacies of fucoidan with and without MSC on bone and blood vessel formation, and implant fixation in critical size bilateral femoral gap-implant defect model in sheep; and 2) to evaluate the potential synergetic effects of anti-infection with vancomycin and bone-preserving ability in mouse and sheep models. Thus, the outcomes of these studies will provide evidence on implant fixation and bone regeneration for future application in challenging bone diseases like osteoporosis in animals and clinical trials in patients.

Flow chart of methods

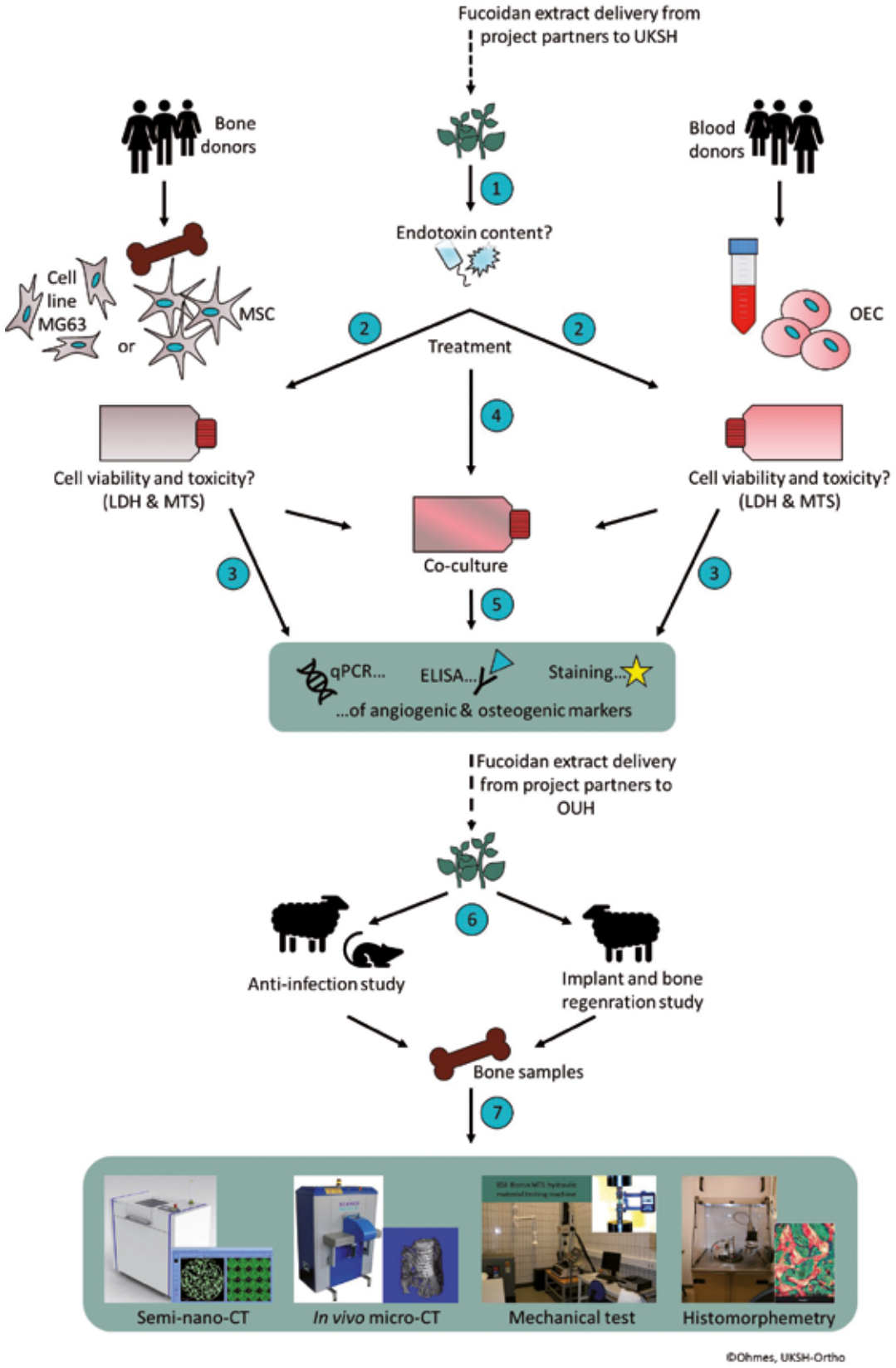

Figure 1: Workflow from University Medical Center Schleswig-Holstein, Department of Orthopaedics and Trauma Surgery (UKSH-Ortho) and Odense University Hospital, Orthopaedic Research Unit, for testing the fucoidan extracts from the FucoSan project in the context of bone regeneration and bone health.

Main findings

The following section provides a summary of the methodology and main experimental findings concerning fucoidan extracts in our models. As described in the previous work packages, algae were collected and fucoidan was isolated by the FucoSan partners Coastal Research & Management oHG (CRM), Kiel University (CAU), University of Southern Denmark (SDU) and Technical University of Denmark (DTU). A high solubility of the extracts in water was the first requirement for the experimental procedure. In addition to the extracts isolated by the project partners, we tested the biological activity of reference substances, including the commercially available crude fucoidan from *Fucus vesiculosus* (Sigma Aldrich). The flow chart in Figure 1 depicts the experimental process to evaluate fucoidan extracts for bone tissue engineering or bone health applications. As the process of algae growing, harvesting and fucoidan extraction has the potential for bacterial contamination, we first tested the endotoxin content of the extracts (Figure 1, step 1). Endotoxins are known to induce severe inflammatory processes and represent a biological risk. Accordingly, only endotoxin-free extracts were further evaluated. As initial screening tools to assess the biological effects of fucoidans on the bone, we used human stem cells such as a) mesenchymal stem cells, isolated from femoral heads and differentiated towards osteoblast-like cells (MSC), and b) outgrowth endothelial cells (OEC) isolated from human blood. Endothelial cells are highly relevant for blood vessel formation throughout the body. In addition, they play a central role in mediating inflammatory reactions.

To test the tolerance of human primary cells regarding different fucoidan doses, we treated the cells with concentrations ranging from 1-200 µg/ml and performed LDH and MTS assays on day one, three and seven after treatment (Figure 1, step 2). We observed that fucoidan treatment slightly decreased metabolic activity in MSC in a concentration-dependent manner, while the metabolic activity of OEC was not affected. Fucoidan extracts did not negatively influence the LDH release in both cell types. In the next step, we investigated the effects of fucoidan on key molecules and molecular markers involved in angiogenesis and osteogenesis in OEC and MCS mono-cultures. In this context, we quantified the gene expression and protein levels of angiogenic and osteogenic markers like vascular endothelial growth factor (VEGF), angiopoietin-1 and -2 (ANG-1, ANG-2), stromal-derived factor 1 (SDF-1) as well as the osteogenic differentiation marker alkaline phosphatase (ALP) (Figure 1, step 3). In order to assess the functional parameters of angiogenesis and osteogenesis and the cellular crosstalk in close approximation to the processes in vivo, we co-cultured OEC and MSC and quantified the above-mentioned angiogenic and osteogenic markers. Additionally, we visualised and quantified the effect of fucoidan on the formation of angiogenic tube-like structures using immunocytochemistry (Figure 1, step 5). We showed that all high molecular weight (HMW) fucoidans tested exhibited anti-angiogenic and anti-osteogenic activities in OEC and MSC mono- and co-culture systems. We found that the observed effects were more pronounced when purer fucoidans rich in fucose and sulphates were applied. This data was similar to the anti-angiogenic and anti-osteogenic properties of the commercially available crude fucoidan reference substance (1). The visualisation of angiogenic tube-like structures in the co-culture system supported our findings based on the quantification of angiogenic markers. Figure 2 shows in two exemplary microscopy pictures how fucoidan treatment reduced the formation of angiogenic tube-like structures.

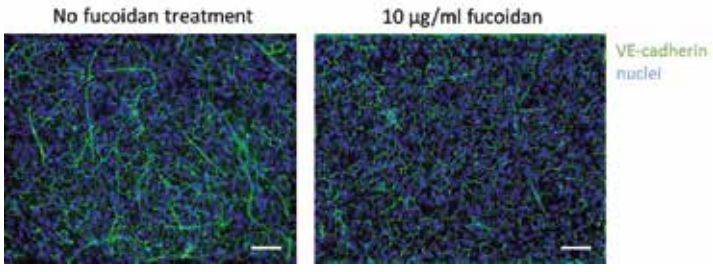

Figure 2: Effect of fucoidan on angiogenic tube-like structures in OEC-MSC co-culture. The adherent's junction molecule VE-cadherin in endothelial cells (green) and nuclei (blue) indicate vascular structures. Scale bar=100µm.

In summary, high molecular weight (HMW) fucoidans derived from different algae species and extracted with different methods negatively influenced angiogenic processes in our in vitro test systems. This effect seems to be mediated by the high affinity of these fucoidan extracts to VEGF. Although anti-angiogenic properties are not in favour of using HMW fucoidans for bone repair, in vivo data is necessary before drawing final conclusions. On the other hand, these HMW fucoidans might be suitable to act as delivery systems for VEGF in bone repair or might be used to limit vascularisation in bone tumours. Based on this data, manuscripts are in preparation (Ohmes et al. and Wang et al.). We are currently testing low molecular weight (LMW) fucoidans to determine if they show different angiogenic and osteogenic effects in comparison to their HMW counterparts.

We are also studying the anti-infection properties of selected fucoidans and performing implant and bone regeneration studies in small and large animal models as stated above (Figure 1, steps 6 and 7).

The outcomes of these studies will provide evidence on implant fixation and bone regeneration for future application in challenging bone diseases like osteoporosis in animals and clinical trials in patients.

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Pilot “Cosmetics”: Introduction of the challenge addressed

The way in which Europe can cope with severe societal challenges regarding marine resources is highlighted by the Blue Growth strategy. However, the exploitation of the sea's abundance of marine living resources will remain underdeveloped unless we create appropriate toolboxes of socially and ecologically responsible (bio)technologies. Indications of such significant gaps include, for example, poor development of fishery management, outdated aquaculture techniques and the utilisation of only a narrow range of species - compared to agricultural techniques on land - as well as the fact that only a handful of pharmaceuticals originating from marine biota have so far been introduced on the market. Furthermore, the rich biodiversity and biomass of seaweed along our regional coasts have been neglected with respect to their value to society.

Against this background, the “Cosmetics” pilot aims to demonstrate the sustainable use of algae by developing economically viable extraction methods and manufacturing processes. In particular, with FucoSan we plan to pave the way for a broad use of the marine biomolecule group of fucoidans in cosmetics, on a straightforward and fast track to the market.

The strength of our team lies in the high degree of complementarity and synergy of different fields of knowledge and technologies. We combine sound expertise in marine ecology, algal physiology, industrial processing and product development, and - last but not least - knowledge of market and customer requirements.

The market for bioactive extracts is attractive, since it has grown at an annual rate of 7.2% between 2013 and 2018 to a total of USD 34 billion (MarketsandMarkets), and is expected to grow further at a similar rate after the coronavirus crisis. In the pilot, we focused on the development of algae extracts as bioactive ingredients for cosmetic products. The life cycles of products in the cosmetics sector are comparatively short. For this reason, the market potential in these sectors can generally be exploited through rapid marketing and marketing-oriented diversification. Depending on the target market, marketing is carried out directly by CRM oHG or by the affiliated company oceanBASIS GmbH. The B2B sector in the "ocean actives" division of oceanBASIS GmbH and their natural cosmetics series "Oceanwell" are suitable for this, in which algae-based ingredients play an important, brand-shaping role.

Species of interest include the “bladder wrack” *Fucus vesiculosus* and other brown seaweed.

The challenges were:

(i) to transform results from the other project partners into economically viable industrial-scale extraction, since extraction of pure fucoidan in the laboratory is complex and costly;

(ii) for the cosmetics market and successfully entering the market, there is a need for higher volumes (minimum: hundreds of kilogrammes per year), that cannot be satisfied by the low quantities from the partners' laboratories, which only produce very small yields (milligrams to grams), and therefore,

(iii) we had to develop an own industrial-scale extraction method, as well as an extract containing fucoidan which offers attractive product features.

Motivation for choosing fucoidans

The aim is to extract the characteristic ingredients of the brown algae genus Fucus, in particular the fucoidans, which are suitable for use in cosmetics.

Fucus species are among the most widespread and abundant macroalgae in the North Atlantic; they have been used for centuries to produce iodine and food, and for medicinal purposes. However, to date there has been no systematic, environmentally friendly and energy-saving use from regional sources.

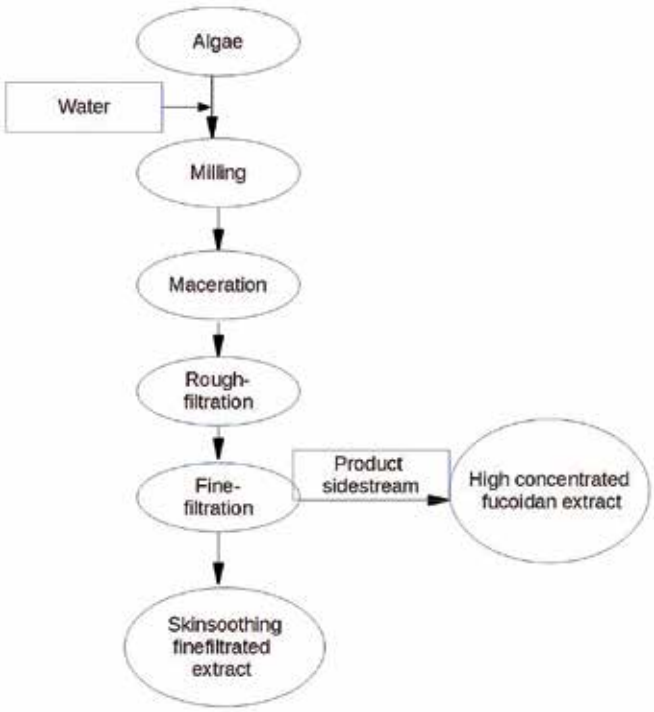
Fucoidans are a complex group of sulphated polysaccharides found in brown algae. As they are highly bioactive, they are increasingly seen as promising candidates for wellbeing, health and medical applications. The broad spectrum of activity of fucoidans is based on the fact that they modulate the diverse (patho)physiological functions of structurally related endogenous glycans. However, their activity profile

can vary considerably, depending on their structural composition and purity, and can even have undesirable effects. Brown algae also occur in the Baltic Sea, but knowledge about the content and composition of their fucoidans has so far been poor. Despite intensive international fucoidan research in the past 20 years, there are only a few fucoidan products available, and no medication yet. One reason is that some peculiarities of these marine polymers, which are relevant for use in products, have long been neglected. A differentiated view of different fucoidans for each specific application therefore plays a central role in marketing, because it offers the key to extensive product diversification.

Accordingly, the development goals are as follows:

- Extraction of fucoidans from regional, sustainably yielded seaweed.
- Investigation of parameters that influence the fucoidan yield and quality, in order to define the optimal sources of brown algae, and to establish protocols for standardised production of an extract containing fucoidan.
- Testing the extracts containing fucoidan in bioassays to generate basic information about their activity and safety profile (cytotoxicity, antiproliferative effects, free radical scavengers, anti-inflammatory effects, testing for usability for cosmetic or dermal applications including any beneficial antimicrobial effects).
- Identification of suitable candidates for product development.

Flow chart of methods



Based on our well-developed commercial extraction methods, we have developed a new extraction method during the project that targets fucoidan.
As shown in the flowchart, the individual steps consist of crushing the algae material (milling), maceration, rough filtration and subsequent fine filtration.
During the sub-process of fine filtration, a by-product is produced which does not pass through the filter.
Investigations of the bypass flow have shown that it contains a high concentration of fucoidan. The challenge in using the off-line extract was the microbiological load. Intensive preservation tests ensured the stability of the extract. The bypass process was optimised during the course of the project, so that a constant quality of the extract is guaranteed. With the newly-developed process, two extracts could thus be obtained during the FucoSan project: a clear extract with skin-soothing properties and a highly concentrated extract with a high content of fucoidan (10.74% fucose in dry matter). The highly concentrated extract is not a specific fucoidan but a total extract with different fucoidan sizes, especially larger molecular units around 280 kDa. Formulation tests have shown that the finely filtered extract is best used as a liquid extract, and the highly concentrated fucoidan extract works better as a dried powder in cosmetic formulations.

Main findings

The results from WP 4, which concentrated on very complex and expensive pharmaceutical and medical use, could not be transferred to the “Cosmetics” pilot on an industrial scale. However, in the market for bioactive extracts for cosmetic applications, it is crucial that the technologies and methods applied are economically and ecologically viable. This implies, in particular, a scalable processing chain as well as an adequate quantity of the resulting product, which is several kilogrammes for a prototype and at least 400 kilogrammes for a first production batch.

Therefore, we developed an extraction process within our work package that is:

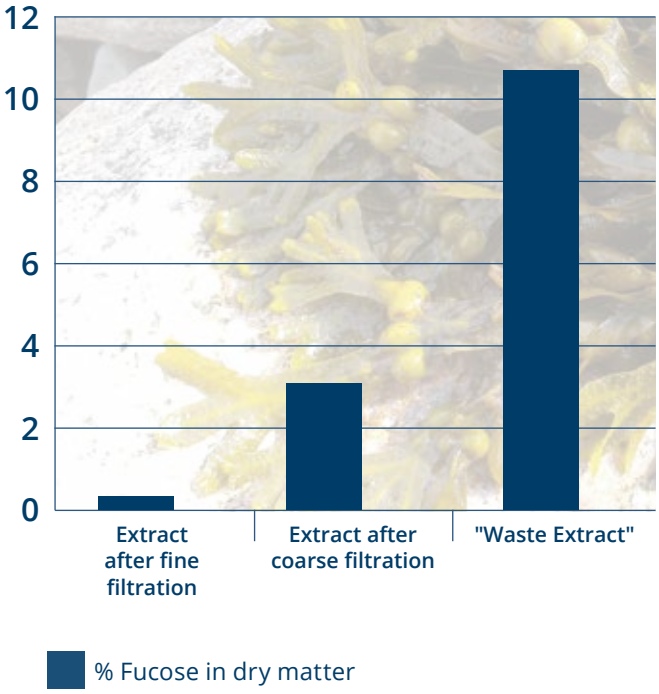
(i) adapted to the specific consistency and chemical composition of the biological raw material, combining optimal crushing, maceration, rough as well as fine filtration and preservation, and,

(ii) suitable for the cosmetics market - in terms of regulation, functional properties and product stability.

According to our product nomenclature, we have named the resulting extract “Fucocrude ‘V”.

Furthermore, investigations of the extract from the side stream have shown that it has a significantly higher content of fucoidan than the finely filtered or roughly filtered extract. This additional processing branch leads to an additional product, which shows that the concept of product diversification by different fucoidan fractions is working – at least for the technical part of the development.

Aqueous Extraction



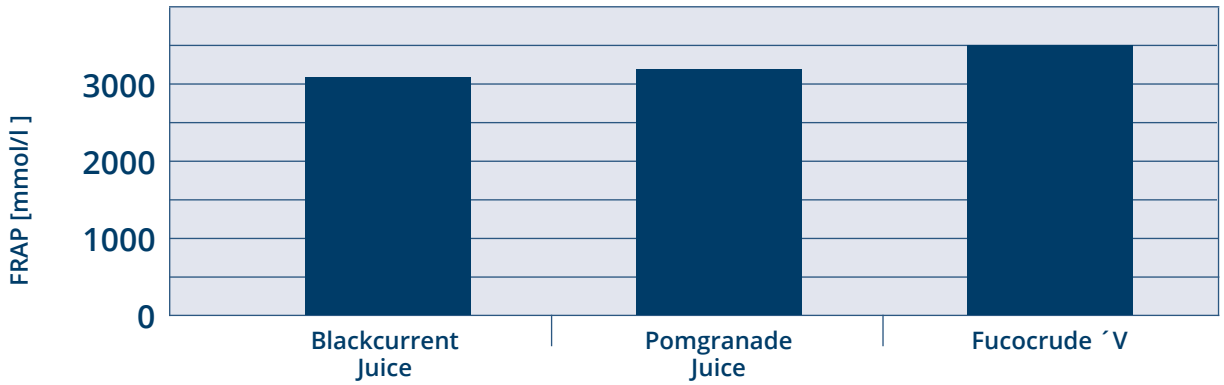
First application tests in a cosmetic formulation could show that the skin of the test persons was strengthened, the perceived skin moisture improved, the effect on the skin is long-lasting, and small wrinkles even disappeared.

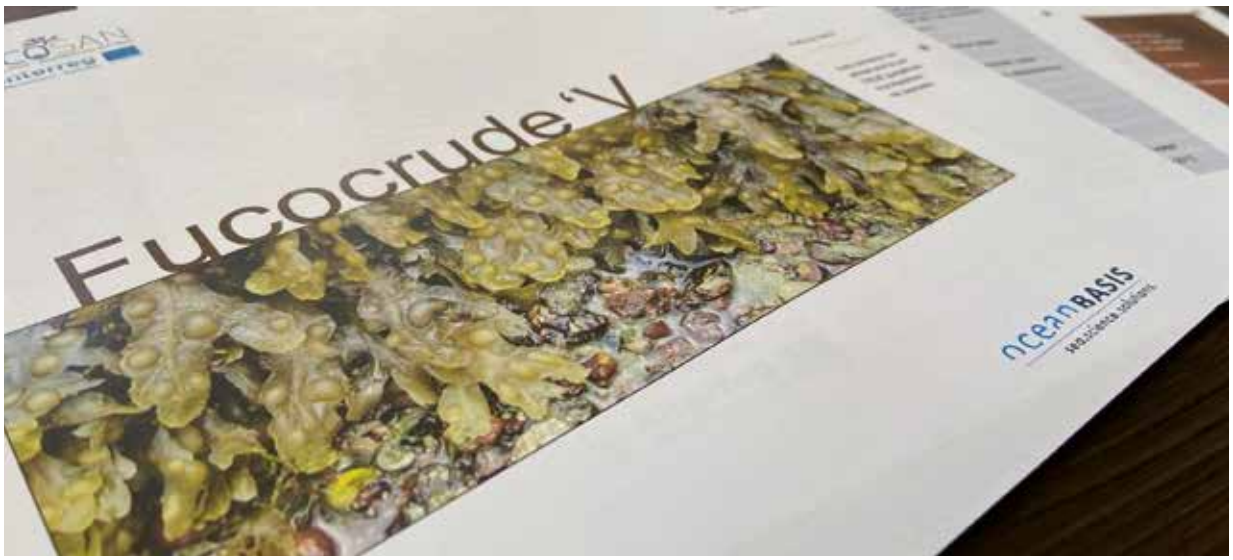
Tests with the finely filtered extract on skin pathogens have shown that this extract has an inhibitory effect on *S. epidermidis*.

	Growth inhibition in %			
Extract Application concentration in %	*S. aureus*	*S. epidermidis*	*Brevibacterium epidermidis*	*Dermabacter hominis*
10	-	44	12	20
5	12	35	23	-
2,5	-	44	17	20
1,25	-	41	19	12
0,5	-	46	11	21

Antioxidant capacity tests showed that the finely filtered extract ("Fucocrude 'V") has a higher antioxidant capacity than other known plant products.

Antioxidative Capacity





Fucocrude 'V – Product specification, safety data sheet and B2B-brochure.

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