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ABSTRACT

Background: Although new therapeutic approaches for burn treatment have made progress, there is still need for better methods to enhance wound healing and recovery especially in severely burned patients. Nanofibrillar cellulose (NFC) has gained attention due to its renewable nature, good biocompatibility and excellent physical properties that are of importance for a range of applications in pharmaceutical and biomedical fields. In the present study, we investigated the potential of a wood based NFC wound dressing in a clinical trial on burn patients. Previously, we have investigated NFC as a topical functionalized wound dressing that contributes to improve wound healing in mice.

Methods: Wood based NFC wound dressing was tested in split-thickness skin graft donor site treatment for nine burn patients in clinical trials at Helsinki Burn Centre. NFC dressing was applied to split thickness skin graft donor sites. The dressing gradually dehydrated and attached to donor site during the first days. During the clinical trials, physical and mechanical properties of NFC wound dressing were optimized by changing its composition. From patient 5 forward, NFC dressing was compared to commercial lactocapromer dressing, Suprathel® (PMI Polymedics, Germany).

Results: Epithelialization of the NFC dressing-covered donor site was faster in comparison to Suprathel®. Healthy epithelialized skin was revealed under the detached NFC dressing. NFC dressing self-detached after 11–21 days for patients 1–9, while Suprathel® self-detached after 16–28 days for patients 5–9. In comparison studies with patients 5–9, NFC dressing self-detached on average 4 days earlier compared with Suprathel®. Lower NFC content in the material was evaluated to influence the enhanced pliability of the dressing and attachment to the wound bed. No allergic reaction or inflammatory response to NFC was observed. NFC dressing did not cause more pain for patients than the traditional methods to treat the skin graft donor sites.

Conclusion: Based on the preliminary clinical data, NFC dressing seems to be promising for skin graft donor site treatment since it is biocompatible, attaches easily to wound bed, and remains in place until donor site has renewed. It also detaches from the epithelialized skin by itself.

1. Introduction

Wound healing process represents a complex series of biological events to restore skin barrier function, prevent dehydration and reduce the risk of bacterial infection. However, delayed wound healing is frequent and may lead to chronic inflammation, especially in burn patients with additional systemic impairments [1]. Currently worldwide, burn wound and skin graft donor site treatments vary widely, and a large number of different wound dressing materials are available for their treatment [1,2].

Careful wound care is a prerequisite in avoiding infection in burn treatment. Repeated, painful dressing changes in burn wound areas and skin graft donor sites often need general anesthesia in the initial stage. There are myriad of different wound dressings, but none of them fulfills all the needed requirements. Therefore and especially when population ages, and incidence of chronic wounds and their risk factors are increasing, there is an acute need for new advanced wound care materials that would be applicable also for other types of wounds such as pressure ulcers.

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Wound dressings have been developed from both natural and synthetic materials. The development of ideal wound dressing material is focused on the requirements of elasticity, moisture and pH maintenance in the wound environment. The capacity to prevent bacterial contamination and to promote painless and rapid wound healing are also important factors [3,4]. Recently, novel silk biomaterial dressings constituted of nanoscale silk fibers have shown promise for wound care when studied *in vitro* in wounded human skin equivalents or *in vivo* in the treatment of skin wounds in mice [5–7].

Cellulose based polymers are one of the most abundant natural products and have a high potential for biomedical and biotechnology applications [8]. Especially nanofibrillar cellulose (NFC) has attracted attention among researchers during the past years. NFC, which is often used as a parallel term with *e.g.* cellulose nanofibrils, microfibrils or microfibrillar cellulose (MFC) is composed of cellulose fibrils of typically some tens of nanometers in diameter and hundreds of nanometers in length. High specific surface area together with highly hydrophilic nature of NFC make it possible to retain a high amount of water in relation to its dry matter. When dispersed in water, NFC creates a strong hydrogel (Fig. 1A) that can be modified for different purposes such as cell culture scaffold in 0.5% concentration (Fig. 1B) or wound dressing manufacturing. NFC derived from wood or plant serves as an animal and human origin-free biomaterial. That makes it interesting in various applications of human care since the regulatory agencies at Europe, USA and Japan are looking for the xeno-free biomaterials to be used at patients' care. Physical and mechanical properties of NFC along with biocompatibility promote its widespread application potential in pharmaceutical and biomedical areas, such as in drug delivery, foams and aerogels, drug and cell carriers, biomaterial substitutes, and scaffold synthesis [9–14]. Wood based NFC hydrogels have shown potential for the formation of three dimensional scaffolds for cell cultures supporting pluripotency of stem cells, differentiation of liver progenitors and formation of organoid spheroids [15–18]. NFC, having a variety of advantages including strength, non-cytotoxicity and ability to maintain moisture, is also highlighted as a promising material for clinical wound healing applications [13,19–22]. Irrespective of promising results *in vitro* and in animal studies, clinical studies concerning the use of NFC in wound healing applications with patients are lacking. A few studies have shown that bacterial growth is impaired or not supported in the presence of nanocellulose [20,23], and also the antiviral effects of the crystalline nanocellulose have been reported [24]. These antimicrobial properties would be an advanced characteristic for a wound dressing.

Different from NFC, carboxymethylcellulose (CMC) hydrofiber dressing with integrated ionic silver has been widely studied as a promising wound dressing material in treatment of burns and ulcers [25]. However, application of CMC dressing in wound treatment usually requires frequent changes of the dressing, which increase the pain burden on a patient [26]. Further, the use of silver has introduced concerns with respect to cytotoxicity [25,27,28]. Bacterial cellulose (BC) or microbial cellulose, on the other hand, has the same sugar-molecule structure as NFC but is biosynthesized by certain bacterial species. It cannot be remodified into different types of phases like hydrogels and wires as wood based NFC since to date there exist no effective production methods to produce strong bacterial cellulose based materials. BC hydrogel has been considered as rather weak due to the swelling property in comparison with many other natural hydrogels [29]. BC mimicking native extracellular matrices performs especially as a promising scaffold material for tissue engineering and for guided tissue regeneration [30,31]. BC as a wound dressing has been shown to reduce wound closure time in clinical treatments of leg ulcers and skin tears [32–34]. Furthermore, in clinical trials among severely burned patients it enabled fast wound healing and showed a high level of adherence to the wound sites due to its conformability [30,35]. However, BC can be penetrated by epithelial cells and may therefore integrate into the skin tissue [36].

We have previously shown that NFC containing human adipose mesenchymal stem cells (hASC) used as a topical functionalized wound dressing contributes to improve wound healing for nude mice [[37] Unpublished results]. Moreover, absence of immune rejection after treatment and the biosafety of NFC alone or in combination with hASC were confirmed in humanized mice.

In the present study, we aimed to investigate the potential of a wood based NFC wound dressing in a clinical trial among burn patients. NFC dressing was used for skin graft donor site treatment for nine burn patients and compared to the commercial lactocapromer based dressing, Suprathel® (PMI Polymedics Innovations, Germany) at the studies with five patients. During the clinical trials, we had an opportunity to analyse and optimize the physical and mechanical properties of the NFC dressing.

2. Materials and methods

2.1. Materials

2.1.1. Nanocellulose

Chemically unmodified wood based NFC was used for wound dressing manufacturing. The NFC was kindly supplied by UPM-Kymmene Corporation (UPM), Finland. The NFC, *i.e.* cellulose nanofibrils, was isolated from bleached birch pulp fibers with a controlled fibrillation and homogenization process using an industrial fluidizer. After the fibrillation, solids content of the NFC hydrogel is typically 16 g/l. The NFC used in this study was specifically developed for wound healing instead of the hydrogel used for the 3D cell culture [15].

2.1.2. Production of NFC dressing

NFC dressing was supplied in close co-operation with UPM. Two principal kinds of NFC dressings were manufactured: 100% NFC dressing (type 1) and reinforced NFC dressing (types 2 and 3; Fig. 1C). Type 1 dressing was manufactured from the NFC hydrogel by applying filtration technique alike in the *in vivo* animal experiments. Type 1 dressings were used for patients 1–2. Types 2 and 3 NFC dressings consisted of an internal reinforced polyester-viscose based gauze between NFC layers on the both sides of the gauze. Type 3 had a reduced amount of NFC compared to the type 2. Dressing type 2 was used for patients 3–5 and

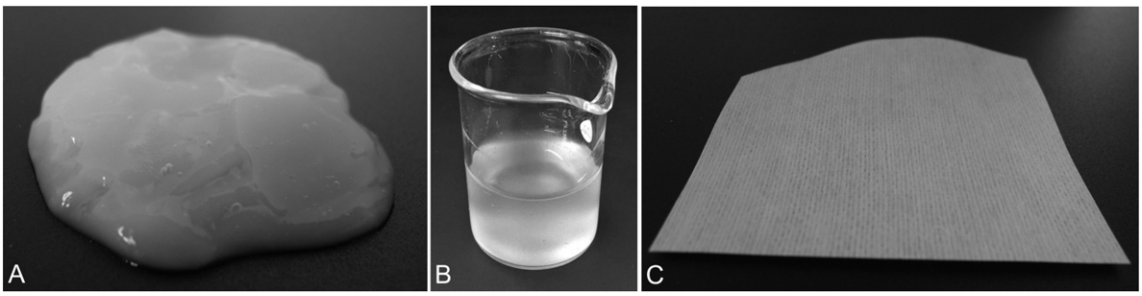

Fig. 1. A) NFC hydrogel used for manufacturing of the NFC dressing. B) Diluted 0.5% NFC hydrogel that can serve as cell scaffold in three dimensional cultures C) Reinforced NFC dressing.

type 3 for patients 6–9. The forming of the all dressings was done by filtrating water. Final water removal was carried out by drying the dressings under pressure. As the 100% NFC dressing, the reinforced NFC dressing was manufactured using filtration technique. All the dressings were cut in shape and packed into sterilization bags. Sterilization was carried out in Systec V-65 autoclave (Systec GmbH, Germany) for 15 min at 121 °C.

2.2. Analysis methods of NFC dressing

Sterilized dressings were air conditioned before measuring their physical and mechanical properties. Air conditioning was done by keeping the dressings in an air conditioned laboratory room at 23 °C temperature and RH50 for at least 12 h. Measurements (except absorption of NaCl solution) were carried out according to standard methods presented in Table 1. 0.9% NaCl solution was produced by UPM from dry NaCl (J.T. Baker, Netherlands) and distilled water.

2.2.1. Grammage of the NFC wound dressing

Grammage (basis weight) was measured by weighing a dressing of a specified area with AG-204 scale (Mettler Toledo, Switzerland). Grammage is calculated by dividing the NFC dressing weight by the area of the measured dressing. Thickness measurement was carried out from a single dressing. Density was calculated by dividing grammage of the dressing with the thickness of the dressing. Thickness measurement was carried out by a thickness measurement device manufactured by Lorentzen & Wettre, Sweden.

2.2.2. Thickness and density of the NFC wound dressing

Thickness measurement was carried out from a single dressing with L&W Micrometer. Thickness is determined as the distance between measurement head and sample tray when sample is placed between them. Density was calculated by dividing grammage of the dressing with the thickness of the dressing. Thickness measurement was carried out by thickness measurement device manufactured by Lorentzen & Wettre, Sweden.

2.2.3. Tensile strength of the NFC wound dressing

Tensile strength was measured by cutting the NFC dressing into 15 mm wide strips and measuring the force needed to break the dressing. 100 mm distance between clamps and an elongation speed of 102 mm/min was used. The tensile tester device L&W Tensile Tester was produced by Lorentzen & Wettre, Sweden.

2.2.4. Tearing strength of the NFC wound dressing

Tearing strength was measured with Elmendorf-tearing strength tester (Lorentzen & Wettre, Sweden). Tearing strength was measured from dressing samples with size of 62 mm × 50 mm. A single sample was used for each parallel (4) measurement. Tensile and tear results are presented as a geometric average of the two main directions (x, y) of the dressing. Geometric average is calculated by multiplying the result of the two main directions and taking a square root of the obtained product of the two measurement values.

2.2.5. NaCl-absorption of the NFC wound dressing

0.9% NaCl absorption was carried out by cutting a dry NFC dressing of a specific area, weighing and soaking the sample in 0.9% NaCl-solution. NaCl-absorption measurement was carried as a series of time (1, 2, 10, 30, 60, 120 and 1440 min). After the selected soaking, the sample was removed from the 0.9% NaCl solution, placed between plotting boards and rolled over once with a steel roller (Lorentzen & Wettre, Sweden) in order to remove the free, non-absorbed 0.9% NaCl solution. Thereafter, the dressing was weighted by AG-204 scale (Mettler Toledo, Switzerland) and put back into the solution for the next soaking period. Results are presented as absorbed NaCl solution amount per square meter of dressing. The used measurement method was modified from the standard method described in ISO 535:2014.

Table 1
Standard methods used for measurement of NFC dressing properties [38].

Grammage	g/m^2	ISO 536
Bulking thickness	μm	ISO 534
Apparent bulk density	kg/m^3	ISO 534
Tensile strength	kN/m	ISO 1924-3
Tear strength	mN	ISO 1974

2.3. In vitro antimicrobial activity of NFC dressing

NFC dressing was evaluated for antibacterial activity against *Staphylococcus aureus* ATCC 25923 (*S. aureus*) and *Pseudomonas aeruginosa* ATCC 27853 (*P. aeruginosa*) using the disk diffusion and macrodilution broth methods according to EUCAST and CLSI guidelines with slight modifications [39,40]. Tests were carried out in aseptic conditions.

For both the disk diffusion and macrodilution broth methods, fresh Mueller-Hinton agar (MHA; BD Biosciences) plate cultures were prepared from bacterial slant cultures and incubated overnight at +35 ± 1 °C. NFC dressing samples were prepared by cutting disks of similar size as of the antibiotic disks (diameter 9 mm) from the sterile dressing. In disk diffusion assays, penicillin (10 units/disk; Rosco Diagnostica A/S) and ceftazidime (30 μg/disk; Rosco Diagnostica A/S) were used as positive controls against *S. aureus* and *P. aeruginosa*, respectively. Fresh colonies of both bacterial strains were used to prepare bacterial suspensions into sterile 0.9% saline. The turbidity of the suspensions was measured by DEN-1B McFarland Densitometer (Biosan, Latvia) and adjusted to 0.5 McFarland standard [39–41] corresponding to 1–2 × 10^8 colony forming units (CFU)/ml. Fresh MHA plates were inoculated with bacterial suspensions by spreading with a sterile cotton swab and the sample disks were applied to the agar plates within 15 min. Plates were incubated at +35 ± 1 °C for 20 h before examination of the results.

For macrodilution broth assays, ciprofloxacin (ICN Biomedicals Inc.) was used as a positive control at four different concentrations in the range of 0.125–1 μg/ml and 0.25–2 μg/ml for *S. aureus* and *P. aeruginosa*, respectively. NFC disks were placed into Mueller-Hinton broth (MHB; BD Biosciences) containing test tubes. Ciprofloxacin control samples were prepared by adding antibiotic stock solution into the control test tubes. Fresh colonies of both bacterial strains were inoculated into sterile 0.9% saline to prepare bacterial suspensions. The turbidity of bacterial suspensions was measured with densitometer and CFU/ml value was calculated. Bacterial suspensions were inoculated into test tubes as a final concentration of 5 × 10^5 CFU/ml. MHB alone or with NFC, or bacterial suspension alone were used as negative controls. Suspensions were incubated at +35 ± 1 °C for 16–20 h, 100 rpm before examination of the results. Results were observed as the turbidity of samples that was measured for calculation of CFU/ml value by densitometer. Percentual bacterial growth was calculated by comparing NFC containing bacterial suspension with bacterial suspension without NFC. Bacterial suspension test was performed three times.

2.4. In vivo biosafety studies in animals

Animal studies were performed with 100% NFC dressing at the Centro de Investigación Principe Felipe (CIPF), under the evaluation and approval from the Animal wellbeing and Research committee in the center (Committee approval number: 12-0238, CIPF, Valencia, Spain). In order to evaluate the toxicity of the NFC dressing, a system for animal full thickness wound healing model in Swiss nu/nu mice (Charles River) was used as described by [42]. The animals were housed and closely monitored at the CIPF animal facilities. Sedation was induced by inhalation of isofluorane (2%). Fentanyl (0.05 mg/kg) and morphine (5 mg/kg) were used for the analgesia during the procedure.

Table 2
Patients and the skin graft donor sites.

Patient nr	Age	Gender	Burn type	TBSA (%)[a]	Dressing type	Body part of NFC covered SKDS[b]	SA[c] of SKDS[b] covered by NFC (cm^2)	Body part of the Suprathel® covered SKDS[b]	SA[c] of the SKDS[b] covered by Suprathel® (cm^2)
1	25	Male	Electric	5	1	Thigh (front)	8 × 8		
2	57	Female	Flame	28	1	Thigh (side)	7 × 8		
3	21	Male	Flame	1	2	Thigh (front)	10 × 10		
4	73	Male	Flame	11	2	Flank	20 × 15		
5	66	Male	Flame	16	2	Thigh (right)	25 × 40	Thigh (left)	25 × 40
6	56	Male	Flame	10	3	Thigh (right)	20 × 35	Thigh (left)	20 × 32
7	62	Male	Flame	47	3	Back	50 × 25	Back	50 × 15
8	56	Female	Hot water	23	3	Thigh (right)	15 × 20	Thigh (right)	15 × 10
9	41	Male	Hot water	10	3	Back	30 × 45	Both thighs	15 × 25

[a] TBSA = total body surface area.
[b] SKDS = skin graft donor site.
[c] SA = surface area.

Once anesthetized, two ipsilateral injuries were performed in the back of each animal by using a surgical punch with 5 mm of diameter (BBraun, Spain). One of the injuries was used as control and the other was covered with the NFC dressing. The number of animals was n = 6. Ten days after the surgery, animals were sacrificed by perfusion through the left ventricle with the aim of preserve all the body. Biopsies from the injured area were embedded in paraffin and processed for sectioning. The pathological evaluation of the samples was performed by an independent pathologist.

2.5. Clinical studies of skin graft donor site treatment

2.5.1. Clinical study and patients

Clinical studies with NFC dressing in skin graft donor site treatment among burn patients were performed at Helsinki Burn Center, Helsinki University Hospital, Helsinki, Finland, from January 2015 to March 2016. During the clinical studies, the properties of NFC dressing were developed further based on the clinical experiences. Nine burn patients, seven men and two women, needing skin grafting were enrolled in this study. Average age of patients was 51 years (SD 18), range between 21 and 73 years. Burn injuries were caused by flames (n = 6), electricity (n = 1) and hot water (n = 2). TBSA% (Total Body Surface Area) values are presented in Table 2. Patients were selected by a plastic surgeon based on clinical evaluation. Exclusion criteria were pregnancy, age under 18 and over 75 years and systemic cortisone treatment. The Research Ethics Committee at the Helsinki University Hospital (HUH) (99/13/03/02/2014) approved the clinical study. All the enrolled patients or their legal representatives gave written informed consent before surgery.

Skin graft donor sites among the first patients (patients 1–2) were small, 8 × 7 cm^2, since this was the first clinical study to test NFC wound dressing in patients. Later, the sizes of skin graft donor sites were up to 50 × 25 cm^2. The accurate sizes of all skin graft donor sites are presented in Table 2. The physical properties of the material were developed based on the feedback from clinics throughout the entire study. Attention was especially paid to the usability of the dressings.

For patients 1–4, NFC dressing was tested alone in skin graft donor site treatment, but from patient 5 forward NFC dressing was compared with the commercial synthetic copolymer of polylactide, trimethylene carbonate, and e-caprolactone, Suprathel® (PMI Polymedic Innovations, Germany), which is regularly used in skin graft donor site treatment at Helsinki Burn Center. Patients' 5 and 6 skin grafts were harvested from both thighs in equal size; the right thigh was covered by NFC dressing and the left thigh by Suprathel®. For patient 7, the NFC wound dressing was placed on large donor site area in the back of the patient while on previous operation seven days earlier, the other skin graft donor site in the back of the same patient was covered with Suprathel®. Donor site of patient 8 was operated in right thigh, and half of the donor site was covered by NFC wound dressing and the other half by Suprathel®. For patient 9, NFC wound dressing was placed on skin graft donor site in the back, while Suprathel® was used in both thighs. After discharge, the patients were followed up in the outpatient clinic up to 11th–75th postoperative day.

2.5.2. The in vivo treatment protocol with NFC dressing

Split-thickness skin grafts were harvested by a plastic surgeon using a Zimmer® air dermatome (Zimmer Inc., Switzerland). Skin graft thickness was 8/1000 in. (0.20 mm), except with the patient 7 where the thickness was 10/1000 in. (0.25 mm). Hemostasis was achieved using adrenaline solution soaked Telfa® gauzes (Medtronic, Switzerland). Before placing NFC dressing on the donor site, it was soaked in 0.9% NaCl solution. NFC dressing was covered by Jelonet® (Smith & Nephew, UK) and fixed with staples (Fig. 2). Dry dressings were used as the outermost covering material. Suprathel® was covered in the same way as NFC dressing but it was not immersed in 0.9% NaCl before placing on the donor site. Dressings were not changed during the entire treatment period.

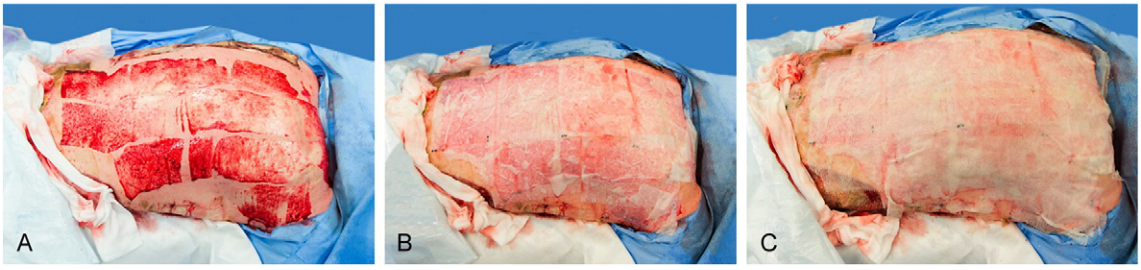

Fig. 2. Treatment protocol with NFC wound dressings in skin graft donor site treatment. A) Skin after harvesting of 0.25 mm grafts. B) NFC wound dressings placed on the skin graft donor sites. C) Donor sites and NFC covered by Vaseline gauze Jelonet®. NFC and Vaseline gauze are attached to skin by staples and covered by dry dressings. ©T Hanski, P Hatanpää, S Rajander, HUS.

2.5.3. Data collection from the clinical use of NFC dressing

We investigated the healing of the donor site that was determined by the self-detachment of material from the skin graft donor site. Designation was based on material property to detach itself from the skin graft donor site after the skin was epithelialized. During the clinical treatments, NFC dressing material was evaluated in terms of strength and pliability. NFC wound dressings were checked by visual observation without changing the material at intervals of few days until the self-detachment, and the photos were collected during the examination of donor sites and wound dressings throughout the clinical NFC dressing periods. Evaluation of the skin condition and epithelialization was done by a burn surgeon. In addition, the possible adverse effects were evaluated and subjective pain experience was asked from the patients.

3. Results

3.1. Characteristics of NFC wound dressing

NFC dressing properties were measured according to the methods presented in Table 1. Grammage, thickness and density describe basic physical properties of the dressings whereas tensile and tearing strength present the mechanical properties and ability of the dressing to resist fracturing. Tensile strength presents how the dressing withstands forces affecting in the direction of the plane of the dressing. Tearing strength measures the out-of-plane strength of the dressing. Strength is needed *e.g.* to resist the forces caused by fixing the dressing with staples. NFC wound dressing properties are presented in Table 3.

100% NFC dressing with grammage of 61,5 g/m2 was used for the patients 1–2. 100% NFC dressing produces a high density and a high in-plane tensile strength. However, this dressing was too brittle to resist fracturing at fixing points of staples.

For patients 3–5, a reinforcement gauze was introduced and the total amount of NFC was simultaneously reduced from 100% to 60% of the total weight of the dressing. The effect of the gauze can be seen as greatly increased thickness of the dressing and resulting in lower density of the dressing. For patients 6–9, the NFC amount in the dressing was further reduced to 50% of the total weight of the dressing in order to speed up wetting and to make the dressing more conformable.

The grammages of the NFC dressing type 1 (patients 1–2) and reinforced NFC dressing type 3 (patients 6–9) were close to each other. Because of the reinforcement gauze, the amount of the NFC per square meter in the reinforced NFC dressing was, however, almost 50% lower than that in the NFC dressing used in patients 1–2. A slight decrease in in-plane tensile strength was noticed due to the lower amount of NFC whereas a major increase in tearing strength was seen. No fracturing of the dressing was seen after the reinforcement gauze was introduced.

The thickness of the gauze largely determines the thickness level of the dressing, which can be seen with no difference in the thickness of the dressing used for patients 3–5 and 6–9. Thickness was measured from the dry dressings. Thickness of wetted dressings has not been measured but fluid absorption affects the thickness.

Table 3
The properties of NFC wound dressing.

Dressing properties/dressing type, patients	1, 1–2 (STD)	2, 3–5 (STD)	3, 6–9 (STD)
Reinforcement gauze	No	Yes	Yes
Grammage of dressing/m^2	61.7 (0.6)	78.5 (5.7)	66.2 (4.45)
Grammage of reinforcement gauze/g/m^2		32.5	32.5
NFC grammage in dressing/g/m^2	61.7	46	32.5
Thickness/μm	58 (2)	130.5 (5.1)	150 (1.4)
Density/g/cm^3	1073 (40)	618 (35)	428 (6.8)
Tear strength, geom ave/mN	63 (1.7)		2308 (381)
Tensile strength, geom ave/kN/m	3.6 (0.9)		2.8 (0.29)
Saline absorption @ 2 min/g/m^2	38.4 (7.4)	91.0 (9.5)	77.3 (6.2)
Saline absorption @ 2 min/g/g	0.62 (0.10)	1.05 (0.04)	1.11 (0.04)

NaCl absorption was measured in order to describe the capability of the dressing to absorb fluids. Based on the absorption measurements carried out, it was seen that the initial wetting of the NFC dressing was very fast and took place within one minute. Thereafter the absorption speed reduced but the NFC dressing was still able to absorb after 24 h of soaking. The amount of absorbed NaCl in the three different NFC wound dressings is presented in Fig. 3.

100% NFC dressing used for patients 1–2 absorbed about 50% less than the NFC dressings used for patients 3–5 and 6–9, in which the amount of NFC was reduced from 100% to 60% and 50% of the total weight of the dressing. The higher amount of NFC in the dressing used for patients 1–2 should have given a higher absorption capacity but it seemed that the combination of gauze and NFC used for patients 3–9 did provide favorable conditions for absorption. It is also supposed that the higher NaCl and fluid absorption capability of NFC dressing improved the attachment of the dressing to wound bed.

NFC dressings used for the patients 3–5 absorbed NaCl and body fluids lightly more than the NFC dressings used for the patients 6–9. This is most probably due to the higher NFC content in the NFC dressing. However, the reduced NFC amount in the NFC dressing used for patients 6–9 made the dressing more pliable, which was beneficial from the NFC dressing usage point of view.

3.2. The antimicrobial activity of NFC dressing

NFC dressing was tested for antibacterial effect against Gram-positive (*S. aureus*) and Gram-negative (*P. aeruginosa*) bacteria by disk diffusion and macrodilution broth methods. No zones of growth inhibition against these bacteria were observed around the NFC dressing disk (the zones observed for positive controls were according to CLSI guidelines). According to our results based on bacterial suspension assays, the presence of NFC dressing in the culture media did not alter significantly the growth of *S. aureus* and *P. aeruginosa* compared to control suspension containing only bacteria.

3.3. In vivo animal biosafety assessment

To analyse any toxic effect of the material after contact with an open injury, the NFC dressing was evaluated using a Swiss nu/nu mouse full thickness skin wound healing model (Fig. 4A–C). In all the cases, the 100% NFC dressing, the same one used with the patients 1–2, detached itself around day 8–9. Histopathological evaluation of the injured area performed by an independent pathologist demonstrated the absence of pathological response. The anatomical structure and appearance of the injured areas in contact with the NFC dressing and their correspondent controls were similar (Fig. 4D and E, respectively). No evidences of cell necrosis, granulomas or giant cells were founded in any of the cases

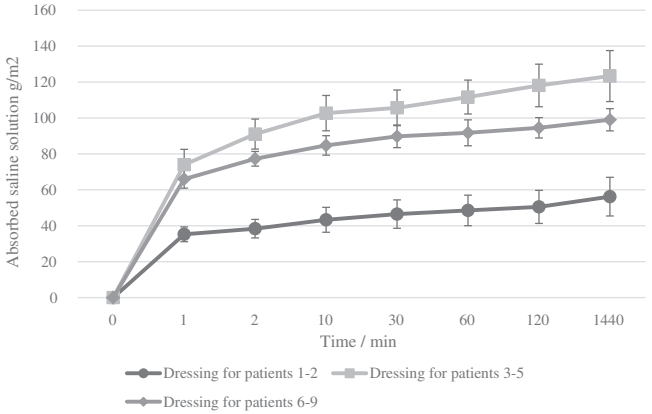

Fig. 3. The amount of absorbed 0.9% saline in NFC wound dressings.

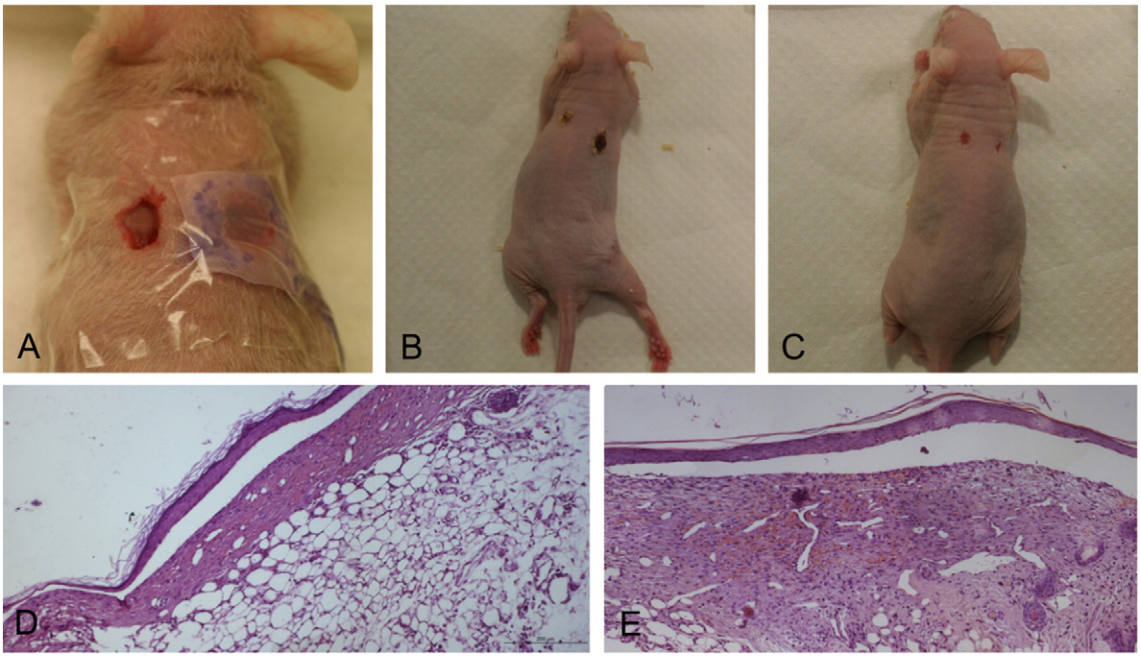

Fig. 4. Full thickness nude mice model and the histopathological analysis of the effect of NFC dressing at the site of injury. A) Coverage and sealing of control and treated area after the injury induction. B) Aspect of both control and treated (dressing-covered) injuries (5 POD). C) Evolution of the area at day 10. The NFC dressing covering the wound area detached itself from the animals after 8–9 days. D) Photomicrography of the control injury stained with hematoxylin and eosine ten days after the surgery (Scale = 200 μm). E) Photomicrography of the injury treated with NFC dressing ten days after the surgery. No signals of giant cells, granuloma, tumor or eosinophils were detected in the animals (Scale = 200 μm).

(Fig. 4E). The presence of eosinophils in the area was also discarded, highlighting the lack of allergic reaction by NFC contact. Absence of biochemical response and inflammation has also been assessed in humanized mice, and these results with results of Swiss nu/nu animals have been described in detail in [[37] Unpublished results].

3.4. Clinical studies of skin graft donor site treatment

After the animal *in vivo* safety studies of the NFC dressing were confirmed in mice, the first *in vivo* clinical tests in skin graft donor site treatment were initiated.

Epithelialization rate of skin graft donor sites under both of the NFC dressing and the Suprathel® were evaluated by determining the postoperative day when the material self-detached from the skin graft donor site and the healthy epithelialized skin was revealed. Detachment of the NFC dressing from epithelialized skin graft donor site is presented in Fig. 5. Among patients 1–9, epithelialization of the skin graft donor site with the NFC wound dressing took 16 days as an average (range between 11 and 21, STD 4). In comparison studies (patients 5–9), NFC self-detached on average on 18th postoperative day (range between 13 and 21, STD 3), while Suprathel® self-detached on 22nd POD (range between 16 and 28, STD 4). Compared to Suprathel®, NFC dressing self-detached few days earlier in 4 cases out of 5. In one case, the materials self-detached at the same day. Self-detachment dates are presented in Table 4. There were no major complications related to the use of NFC.

When the NFC dressing trials started with the first patient, the used 100% NFC dressing was very stiff and fragile. Therefore attention was paid to develop a more pliable and stronger NFC dressing, which was achieved along with the clinical tests with the patients for the skin graft donor site treatment. With the patient 1, the stiffness and fragility of NFC dressing, which was 100% of NFC, did not cause any harm for the patient, and the skin graft donor site healed well within 17 days. With the patient 2, the NFC dressing treatment was interrupted at the first postoperative day due to premature detachment of the NFC dressing probably because of the too high stiffness of the NFC dressing.

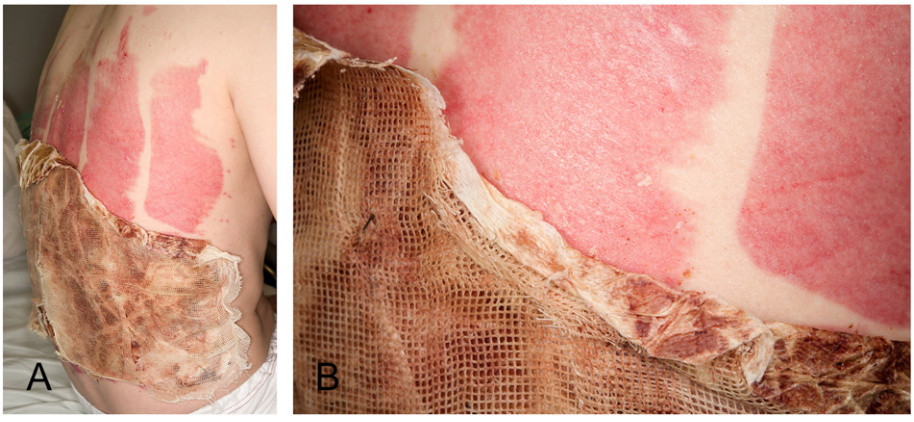

Fig. 5. NFC wound dressing in skin graft donor site of patient 9 on 13th POD. A) Figure shows a special characteristic of NFC wound dressing: it self-detaches the epithelialized skin graft donor site. B) Figure zoomed from Panel A. ©T Hanski, P Hatanpää, S Rajander, HUS.

Table 4
Detachment days of the wound dressings (postoperative days).

Patient nr	NFC dressing type	Body part of NFC covered SKDS[a]	SA[b] of SKDS[a] covered by NFC (cm^2)	NFC detachment POD[c]	Body part of Suprathel® covered SKDS[a]	SA[b] of the SKDS[a] covered by Suprathel® (cm^2)	Suprathel® detachment POD[c]
1	1	Thigh (front)	8 × 8	11			
2	1	Thigh (side)	7 × 8	Discontinued			
3	2	Thigh (front)	10 × 10	11			
4	2	Flank	20 × 15	Discontinued			
5	2	Thigh (right)	25 × 40	17	Thigh (left)	25 × 40	22
6	3	Thigh (right)	20 × 35	17	Thigh (left)	20 × 32	21
7	3	Back	50 × 25	21	Back	50 × 15	28
8	3	Thigh (right)	15 × 20	21	Thigh (right)	15 × 10	21
9	3	Back	30 × 45	13	Both thighs	15 × 25	16

[a] SKDS = skin graft donor site.
[b] SA = surface area.
[c] POD = postoperative day.

100% of NFC dressing was reinforced by applying a gauze in the dressing structure. The reinforced NFC dressing used for patients 3 and 4 appeared to be strong and pliable. No problems were reported in case of the patient 3. For the patient 4, the donor site treated with NFC dressing was infected. However, with the same patient 4, the infection was present also in another donor sites as well as in burned areas treated with different materials. Therefore, the clinical test with the patient 4 was discontinued due to the infection.

The first comparisons with the different wound dressing materials were performed with the patient 5, for which both NFC dressing and Suprathel® was applied. According to the self-detachment dates and the clinical evaluation concerning skin condition, NFC dressing during skin graft donor site treatment performed better than Suprathel®. On the 75th POD, skin condition in donor site covered by NFC dressing did not differ from the donor site treated by Suprathel®. The stiffness of the reinforced NFC wound dressing was still concerned to be too high.

Among the patients 6–9, NFC dressing was developed further towards better usability. No problems were recorded for patient 6 due to the use of NFC wound dressing. For the patient 6, NFC dressing self-detached the skin graft donor site area completely four days before Suprathel®. In the case of the patient 7, NFC dressing self-detached from the donor site seven days earlier than Suprathel® but after the detachment of the materials, skin tears were revealed under both NFC dressing and Suprathel® (Fig. 6). In the case of patient 8, both of the materials self-detached from the donor site at the same day (Fig. 7). However, few days after materials had detached, inflammation was noticed in the donor site covered by Suprathel® (Fig. 7C). In the case of patient 9, NFC dressing performed well in donor site treatment in the back: it stayed still in place and detached the donor site on the 13th POD. Suprathel® detached from the thighs of the same patient on the 16th POD.

No allergic reaction to NFC dressings were reported in any of the patients. The reinforced and further modified NFC dressing exhibited the hoped and correct elasticity with appropriate adherence to the wound of the patient. It is also worthy to notice, that the NFC dressing did not cause more pain for patients than the traditional methods to treat the skin graft donor sites.

4. Discussion

Based on these *in vivo* clinical studies with nine patients, NFC dressing seems to be very promising material for skin graft donor site treatment among burn patients. Before the clinical studies in small donor sites, NFC dressing was tested first *in vivo* in nu/nu mice to evaluate the biocompatibility and ensure the absence of any toxic effect triggered by the material. Indeed, no pathological response was observed in mice. After promising primary results with the first three patients, the treatment was expanded into large and challenging skin graft donor site

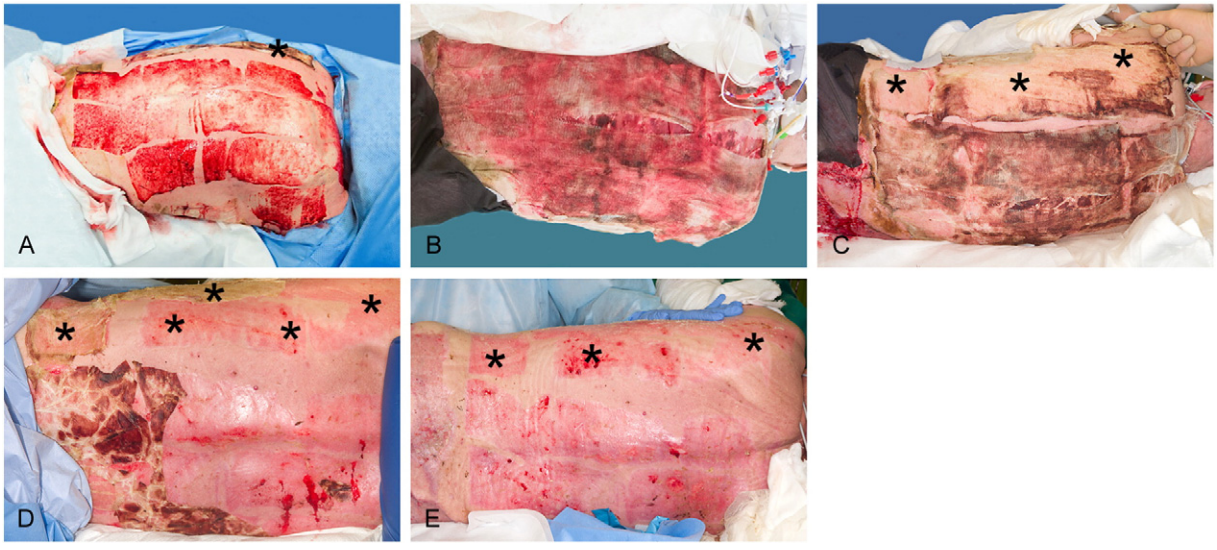

Fig. 6. Skin graft donor site treatment in patient 7. A) Skin graft donor sites in operation. Suprathel® that was placed onto donor site seven days earlier is shown on top with an asterisk. B) NFC wound dressing attached well to the skin. On POD 3, dressings were still moist. C) On POD 7, NFC dressings had dried and were still in place. Donor sites covered by Suprathel® are shown with asterisks. D) POD 15. NFC has partially self-detached from the donor sites, just like Suprathel® (shown with asterisks). E) POD 21. NFC and Suprathel® (marked with asterisks) have self-detached. ©T Hanski, P Hatanpää, S Rajander, HUS.

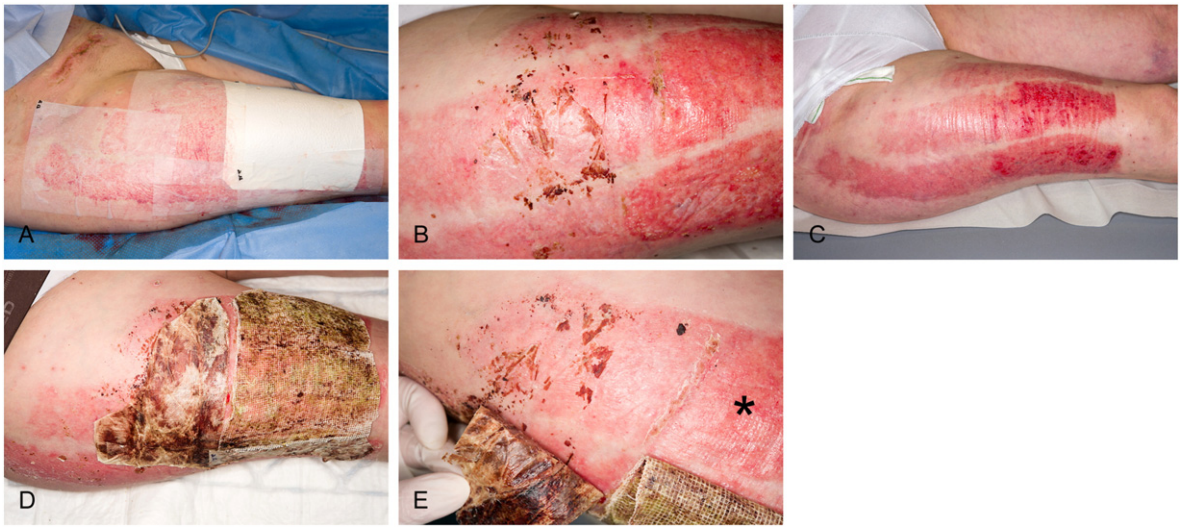

Fig. 7. Skin graft donor site treatment in patient 8. A) NFC dressing (transparent) placed on donor site on the left, Suprathel® (white) on the right. B) Skin graft donor site on dressing detachment day 21st POD. C) Donor site area treated with Suprathel® (on the right) shows irritation. D) Skin graft donor sites and wound dressings on 18th POD. NFC dressing is shown in the left, Suprathel® in the right. E) Epithelialized donor site under the Suprathel® (asterisk) and NFC on 18th POD. ©T Hanski, P Hatanpää, S Rajander, HUS.

areas. Results from these cases further confirmed positive results concerning biocompatibility between the human tissue and NFC dressing. Skin graft donor sites epithelialized well in all patients treated with NFC dressing until its self-detachment.

Essential characteristic for a wound dressing is its ability to absorb secreted wound fluid as well as easy removal from the wound after epithelialization. Gauzes traditionally used as skin dressing materials show large permeability but tight adhesion on the wound bed, inducing unnecessary pain on removal [43]. Both NFC dressing and Suprathel® attached to the wound bed and protected the skin graft donor site. After the skin graft donor site was epithelialized, both materials self-detached from the donor site itself without any discomfort on removal. Based on this, epithelialization rate of the skin graft donor site was evaluated by determining the time elapsed between skin graft harvesting and material detachment from the donor site. According to our comparison studies, NFC dressing self-detaches from the donor site on average of 18 days, while Suprathel® in 22 days. Although n value of our clinical study was small and in all cases the areas were not comparable in terms of anatomical areas, the results were consistent. In the case of the patient 7, NFC dressing was placed on the skin graft donor site in the back while Suprathel® was placed to thighs. In this case, NFC dressing self-detached on 13th postoperative day and Suprathel on 16th. Central sections of the body (back) may heal more efficiently than distal regions (thighs) but on the other hand, more rubbing may be directed to the back compared to thighs. In the cases of the patients 5 and 6, NFC dressing and Suprathel® covered the similar donor site areas; the other material was placed on the right thigh and the other on the left. Also among these patients, self-detachment of Suprathel® took 3–4 days longer than the self-detachment of NFC dressing.

Biocompatibility of NFC has been reported in previous studies [9,15]. According to our clinical experience, NFC dressing did not cause any adverse reactions. However, this must be confirmed in our further planned studies by evaluating the presence of inflammation markers from patient biopsies and blood samples. Although NFC dressing may not show property of tissue repair by itself, it may serve as biocompatible and nontoxic matrix for cells and proteins that contribute to natural repair processes.

Suprathel® has been proved to be biocompatible material, and no allergic reactions are reported [44–51]. In two comparison studies out of five, we registered some irritation in skin graft donor sites covered by Suprathel® after the material was detached. Irritation was not present in the donor site areas covered by NFC dressing (Fig. 7). Phenomenon causing irritation is not clear, but it is a subject to be studied. Initial pH of the Suprathel® is 5.5, and *in vitro* it can decrease down to pH 4. Initial pH of the NFC is typically 6–7. Regulation of the pH value has thought to be beneficial especially in treatment of chronic wounds, which usually show decreased H^+ concentrations. Decreasing the pH may be beneficial due to increase in antimicrobial activity, release of oxygen, and reduction of toxicity caused by bacterial end product [52,53]. Decreased pH leading to alteration in protease function may have positive or negative effects on wound healing, since the balance between matrix metalloproteases (MMPs) and tissue inhibitors of MMPs (TIMPs) is important [54,55]. However, keratinocyte function and re-epithelialization may be impaired due to pH reduction down to 5 [56]. Therefore, it is a subject to be considered, if it is necessary at all to decrease the pH level in the treatment of skin graft donor site.

Regarding the possible antimicrobial properties of wound dressing materials, NFC dressing used in the present study did not significantly affect the growth of common wound pathogens *Staphylococcus aureus* and *Pseudomonas aeruginosa* in bacterial suspension. These results suggest that NFC dressing do not have antibacterial properties but neither does it support bacterial growth. Previously, nanocellulose diluted in deionised water and used as a suspension with bacterial growth medium was shown to reduce the growth of *P. aeruginosa* [20]. Powell et al. also demonstrated an increased potential for NFC to impair bacterial biofilm growth compared to CMC wound dressing material [19]. It may be possible that NFC dressing used in wound care forms a physical water layer-based barrier towards external microoganisms and entraps bacteria within its fibers. If required, antimicrobial activity can be introduced by conjugation of antimicrobial agents such as silver particles (Ag) or antiviral agents like tyrosine sulphate mimetic ligands into the nanocellulose biomaterial [24,57–59].

During the clinical trial, the relationship between NFC content and pliability of the dressing became evident. Among the first patients, the material was too stiff and fragile, but after decreasing the NFC content in the dressing the pliability and adsorption capacity was significantly enhanced. NFC is very hydrophilic thanks to its high amount of hydroxyl groups. Hydrophilicity may contribute to the performance of NFC dressing by forming a water film between the dressing and the wound surface. Our tentative hypothesis is that the water film forms a favorable environment for skin regeneration. In addition, the water film may prevent integration of the dressing into the tissue. Biocompatibility of NFC is important not only in wound dressing applications as such but in offering a possibility to use NFC dressing as a carrier for cells or bioactive

proteins to enhance wound healing. Furthermore, the architecture of NFC having a porous network structure may be useful for potential transportation of antibiotics or other drugs into the wound while providing an effective physical barrier against infections [60].

We have continued to further studies concerning the use of NFC dressing in skin graft donor site treatment. Our aim is to extend the clinical trial to a larger comparison study between NFC wound dressing and Suprathel®. Moreover, further studies will include the collection of biopsies and blood samples from the patients in order to study wound healing and inflammation in skin graft donor sites. In addition, the quality of the epithelialized skin should be determined by a standardized method, and the pain experienced by patients will be evaluated by VAS (visual analogic scale).

5. Conclusion

In the present study, NFC seemed to be highly biocompatible in the treatment of skin graft donor sites. NFC dressing adhered well to the wound bed and detached from the wound surface itself after skin recovery. According to preliminary comparison studies with NFC dressing and Suprathel®, epithelialization under the NFC dressing seemed to be faster compared with Suprathel®. However, further studies are required in order to evaluate the capacity of NFC dressing to promote the wound healing process. In the future, also other forms of complicated wound processes constitute a potential application area for NFC dressing.

Declaration of interest

The writers do not have any personal benefit of the materials and results. All the results are owned by the researchers at the first stage. UPM has kindly sponsored the research and supplied NFC hydrogel and NFC wound dressing. K.L. and M.K. have participated in writing of manufacturing and characterization parts of the NFC hydrogel and NFC wound dressings.

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