## 1. Research Outline

<table>
<thead>
<tr>
<th>Acronym</th>
<th>WOUNDHEALER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project name in English</td>
<td>ROLES AND APPLICATIONS OF BORON COMPOUNDS IN CUTANEOUS BURN AND CHRONIC WOUND HEALING</td>
</tr>
<tr>
<td>Pitch (1 sentence)</td>
<td>Boron containing hydrogel is able to heal burn and chronic wounds effectively.</td>
</tr>
</tbody>
</table>

**Executive summary (max. 10 lines)**

Burn and chronic (non-healing) wounds are the major challenge of current dermatological applications. Therefore, developing new, safe, and effective wound healing drugs has always been an attractive area of international research. In the current study, sodium pentaborate pentahydrate (NaB), pluronics (Plu; F68 and F127), and their combinations were investigated for their wound healing activities, using in vitro and in vivo approaches. The results revealed that NaB significantly increased migration capacity and superoxide dismutase activity in primary human fibroblasts. Therefore, our results suggest that NaB, and its pluronics combination, could be used in dermatological clinics and be a future solution for burn and chronic wounds.
2. Cause and context of the research

After a disruption of skin integrity, the body produces an immediate response followed by a functional and comparable regeneration period, referred to as wound healing. The wound healing process consists of five main orderly but temporally overlapping phases: homeostasis and inflammation, granulation tissue formation, neovascularization, re-epithelialization, and remodeling [1]. These phases are tightly regulated by a cascade of external and internal stimuli such as growth factors and cytokines in a well-orchestrated manner, resulting in regeneration and restoration of the damaged skin [2]. Although normal wounds do not need much attention during the healing period, chronic (non-healing) wounds, experienced by approximately 6.5 million patients in USA alone, are a major challenge of the current dermatological applications [3]. Burn injuries, the most common and destructive forms of wounds, are generally faced with life threatening infections, inflammation, reduced angiogenesis, inadequate extracellular matrix production and lack of growth factor stimulation. In these wounds impose a heavy worldwide social, economic, and health burden due to a lack of efficient wound healing agents. Therefore, developing new, safe, and effective wound healing drugs has always been an attractive area of international research.

Boron has been recognized as an important micronutrient in plant physiology for almost 100 years [4], while limited studies have reported its vital roles in animal and human systems without exploring its exact mode of action. It has been reported to be involved in embryogenesis [5], bone growth and maintenance [6], immune responses [7], hormone action [8], and brain and psychological functions [9] of animal and human metabolism. Besides, it has been shown to increase the wound healing rate in a few studies. Boric acid (3% solution) treatment of deep wounds in an intensive care unit decreased hospitalization time by enhancing granulation tissue formation [10]. Although there are a few similar studies reflecting boron’s positive effects on wound healing, the data supplied in the literature does not sufficiently explore its potential role for use in dermatological clinics. Poloxamers, known as pluronics or kolliphors, are nonionic and amphipathic triblock copolymers consisting of a backbone of poly(ethylene oxide)-b-poly(propylene oxide)-b-poly(ethylene oxide) (PEO-PPO-PEO), which can form micelles and hydrogels at above critical gel concentrations, under proper conditions [11]. These synthetic polymers have been used in a wide array of biomedical areas including medical, pharmaceutical, and the cosmetic industry [12]. They are mainly used in delivery of drugs such as therapeutic proteins, chemicals, cytokines, and antimicrobial agents [13]. Poloxamers have also been used for wound healing agent delivery studies [14, 15]. Other than being used as drug carriers, two important members of this family, F68 and F127, have been shown to be effective in wound healing themselves, by inhibiting inflammation and stimulating growth factor expression [16, 17]. In the present study, we evaluated the effects of a sodium pentaborate pentahydrate (NaB) containing carbopol-based gel composition, co-formulated with poloxamers (F68 and F127) on wound healing both in vitro and in vivo using a full thickness wound model in rats. This is the first study proved excisional wound healing properties of NaB-poloxamer containing hydrogel formulations.

References
3. Innovation results achieved

In the current study, sodium pentaborate pentahydrate (NaB), pluronics (Plu; F68 and F127), and their combinations were investigated for their wound healing activities, using in vitro and in vivo approaches. The results revealed that NaB significantly increased migration capacity and superoxide dismutase activity in primary human fibroblasts. Combinations of optimized concentrations for pluronic block co-polymers further increased cell migration, and the messenger RNA (mRNA) expression levels of important growth factor and cytokines (vascular endothelial growth factor (VEGF), transforming growth factor beta (TGF-β), and tumor necrosis factor alpha (TNF-α)). NaB containing hydrogel co-formulated with pluronics was also investigated for their wound healing activities using a full thickness wound model in rats. Macroscopic and histopathological analysis confirmed that wounds in combination gel-treated groups healed faster than those of control groups. NaB/Plu gel application was found to increase wound contraction and collagen deposition in the wound area. Therefore, our results suggest that NaB, and its pluronics combination, could be used in dermatological clinics and be a future solution for chronic wounds. Studies on burn are in progress.

**Fig. 1** a Cell morphology (Scale bar 400 µm) and b collagen type I immunostaining (Scale bar 100 µm) of human fibroblasts (HF) from neonatal foreskin at passage number 2. The viability of HF cells treated with different concentrations of c NaB, d F68, and e F127 for 24, 48, and 72 h. Data represent the mean values±S.D. Comparisons were performed by ANOVA (Tukey post hoc). *P<0.05, **P<0.01: comparison with control group. NaB sodium pentaborate pentahydrate, NC growth medium-treated HF cells
Fig. 2  

a) Migration rates, b) superoxide dismutase (SOD) activity and relative mRNA levels of c) vascular endothelial growth factor (VEGF), d) fibroblast growth factor 7 (FGF7), e) transforming growth factor beta (TGF-β), and f) tumor necrosis factor alpha (TNF-α) in human fibroblast (HF) cells treated with sodium pentaborate pentahydrate (NaB, 15 µg/ml), F68 (10 µg/ml), F127 (10 µg/ml), F68-F127 (10 µg/ml for each one), and their combinations (Gel). Data represent the mean values±S.D. Comparisons were performed by ANOVA (Tukey post hoc). *P<0.05, **P<0.01: comparison with control group; #P<0.05, ##P<0.01: comparison with F68; §§P<0.01: comparison with F127; †P<0.05, ††P<0.01: comparison with F68-F127; ♦♦P<0.01: comparison with NaB. Control: growth medium-treated HF cells.
Fig. 3 a Photographic representation of wound left untreated (control) or treated with vehicle (hydrogel) and combination hydrogel (Gel). B. Wound contraction rates were expressed as percentages of baseline (n=8), *P<0.05, **P<0.01: control versus hydrogel, ##P<0.01: control versus gel, §P<0.05: hydrogel versus gel. c Wound area was expressed as percentages of baseline (n=8), *P<0.05, **P<0.01. Hydrogel: vehicle carbopol-based hydrogel, gel: 3 % (w/v) sodium pentaborate pentahydrate, 2 % (w/v) F68 and 2 % (w/v) F127 containing hydrogels. Data represent the mean values± S.D. Comparisons were performed by ANOVA (Tukey post hoc).

4. Link to the PRoF value

The overall data suggest that a combination of boron and pluronics increases fibroblast migration, antioxidant enzyme activity, growth factor expression levels, and acute cutaneous wound healing. This formulation could be used in burn and chronic wounds such as trauma, diabetic, decubitus, and venous leg ulcers in order to explore its full potential in dermatological science. Finally, as the formulation provides a remarkable increase in fibroblast proliferation and collagen synthesis, potential use of the formulation for cosmetic purposes should be investigated. The data suggest that boron and pluronics combination can be used to develop some other novel wound healing products such as cream, spray, patch and pads, which are innovating healthcare products for PRoF 2015.
5. Applicable IPR rules

Yes

6. Information on the partner

Prof. Fikrettin Sahin, Mr. Selami Demirci and Ms. Ayşegül Dogan, Department of Genetics and Bioengineering at Yeditepe University, Istanbul Turkey.

Prof. Ertugrul Kılıc, Department of Physiology, Medical School, Istanbul Medipol University, Istanbul, Turkey.

Prof. Erhan Aysan, Department of General Surgery, Medical School, Bezmialem Vakıf University, Istanbul, Turkey.
Addendum: Contact information

Current Address: Prof. Fikrettin Sahin
Yeditepe University
Faculty of Engineering and Architecture
Department of Genetics and Bioengineering
34755 Kayışdağlı
Istanbul/TURKEY

Phone: (216) 578 0935
Fax: (216) 578 0829
E-mail: fsahin@yeditepe.edu.tr