BETA GLUCAN ENHANCES THE WOUND HEALING PROCESS IN ZEBRAFISH

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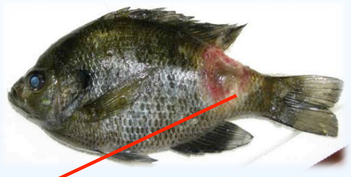
Introduction

β-glucans are natural compounds that interact with the innate immune system. Macrophages play an important role in wound healing process and appear to act as the key regulatory cells for skin repair by removing dead tissues and killing pathogens. Macrophages also produce growth factors that stimulate cells involved in wound healing (production of extracellular matrix). Discovering natural products which may enhance the wound healing process in fish, has numerous health benefits and 1,3-1,6 ß-glucans may reduce the negative effect of stress, inflammatory reactions, and secondary infections.

- Fish produced in aquaculture are subjected to potential tissue damage due to high density stocking.

Tissue damage & wounds

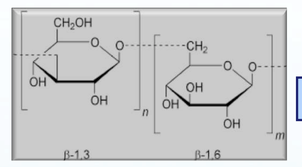
Fighting
Handling
Infections
Virus, bacteria, parasites



Effects of tissue damage on fish

- Slower growth
- Immune suppression
- Secondary Infections

Agents for rapid wound healing



Activation of
- Phygocytosis
- NK cells
- Macrophages
- Lymphocytes
- Complement system

β-glucan
- ✓ Immunomodulator
- ✓ PAMPS
- ✓ β-glucan promotes wound healing by activation of macrophages

Animal model

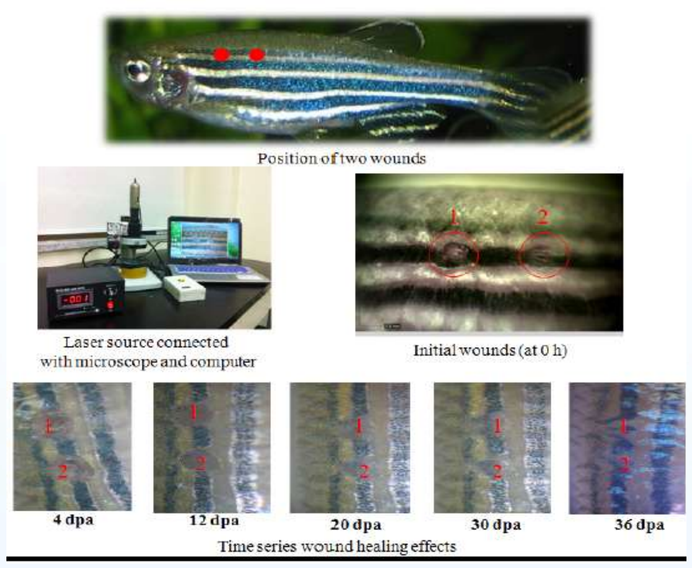
Zebrafish (*Danio rerio*)
- ✓ Low cost model
- ✓ Higher visualization of cellular activity
- ✓ Regeneration ability (fin, heart, etc.)

Major events of wound healing

Inflammation Cytokine action | **New cell proliferation** Growth factors | **Tissue remodeling** Healing

- Wound healing requires the proliferation, differentiation and recruitment of numerous cell types, including keratinocytes, endothelial cells, fibroblasts and immune cells.
- **Objective: to investigate the effect of two MacroGard® products (MI and MII), based on 1,3-1-6 ß-glucans, on wound healing process in zebrafish.**

Materials and Methods



Experimental design

- 90 fish.
- 3 treatments : control, MI and MII.
- MacroGard® dose: 12.5 mg kg^{-1} BW (calculated according to the fish FI, preliminarily estimated).
- Treatment: 2 weeks before injury.

Laser wounding

- Two circular shaped wounds (∅ ~2 mm) were made using laser source (red spots on the picture shown above).
- Wounds were then digitally photographed at 2, 4, 10, 16, 20, 30 days post wound (dpw).
- Wound area was measured by Image J® software.
- Wound size given as a mean of the two wounds for each fish.

Results and Discussion

Table 1: Measure of wound area (mm^2)

group	C		MI		MII		SEM	F value
dpi	n	m	n	m	n	m		
2	25	1.900	27	1.819	27	1.763	0.0700	0.4427
4	29	1.882 A	29	1.725 B	28	1.665 B	0.0641	0.037
12	27	1.146	28	1.076	27	1.042	0.0568	0.3467
16	27	0.994 A	27	0.766 B	27	0.634 B	0.0529	<.0001
20	28	0.702 A	28	0.713 A	27	0.518 B	0.0568	0.0132

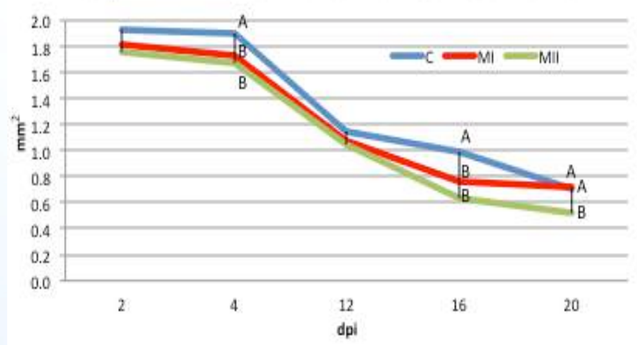
Note: column not connected by the same letter differ per P<0.05

Table 2: Healed fish

dpi		C		MI		MII		Chi^2	P
		healed	not healed	healed	not healed	healed	not healed		
30	n	6	20 B	12	17 AB	19	9 A	11.493	0.0032
	%	23.1	76.9	41.4	58.6	67.9	32.1		

Note: column not connected by the same letter differ per P<0.05

Graph 1: Wound area in relation to treatment and time



- Early wound size differences (1.882, 1.725 and 1.665 mm2) observed at 4 dpw for control, MI and MII, respectively; differences between MI, MII vs control group were statistically (P<0.05) significant.
- Final wound status (at 30 dpw) showed that fish with completely healed wounds were 67.9% for MII group, 41.4% for MI fish and 23.1% only for control group; differences between group MII and control group being statistically significant (P<0.05).

Conclusion

Since results confirmed that 1,3-1-6 ß-glucans may positively affect the wound healing process it is highly suggested their inclusion in fish feeds. Moreover, it seem that the new MacroGard® generation may have a potential enhanced effect on on wound healing process than the present generation (MI).

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