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Extracts of fly-maggots and their effects on the biology of chronic wounds

Results of a series of case studies

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Introduction

Since several decades the larvae of the green botte fly *Lucilia sericata* are again utilized in the so called “biosurgical débridement” of chronic cutaneous wounds. However, it takes an enormous logistic effort to make these maggots available in an excellent quality at the time of use. This makes it very expensive in comparison to other therapeutic strategies of wound management. Furthermore the sensation of free crawling living maggots on the already painful wound area leads not seldom to denial due to the feeling of disgust. In addition, there are some contraindications for maggot therapy such as colonization by bacteria like *Pseudomonas aeruginosa* or the proximity to large blood vessels. One aspect of this study was to investigate, if it is possible to develop a stable, sterile extract of these larvae and thereby circumvent the limitations of the maggot therapy and making it accessible to a higher number of patients.

Method

A method was developed to create heated, sterile whole body extracts of maggots that were made stable by sterile filtration, lyophilization and γ-radiation. These extracts were applied to ulcers of ten volunteering patients for periods of up to eight weeks. The status of the ulcers was documented photographically weekly, screened for bacterial colonization every other week and samples of wound exudates were taken on a weekly basis. Exudates were screened for concentrations of matrix metalloproteinases (MMP) -1, -2, -3, -8, -9, -10 and -13 as well as for tissue inhibitors of metalloproteinases (TIMP) -1, -2 and -4 in order to identify possible diagnostic or prognostic markers of cutaneous wound healing.
Case 1

The 46-year-old female patient suffered from fibrinous layered ulcera crurum since about 1 year. She was non diabetic but additionally suffered from polyarteritis nodosa. The patient complained of intense and permanent pain in the area of ulcers.

Photo board 1: Wounds of patient 1 during the course of treatment with maggot extract. The photos show the inside of the lower right leg of the patient. Wound treatment of the female patient was done three times a week. After the maggot extract has been dissolved, it was dropped onto the wound. Treatment was done in the Clinic for Dermatology of the University Clinics Duesseldorf, Germany. Photos A and B show wounds before the first treatment, photo C after two weeks of treatment, photo D after four weeks, photo E after six weeks and photo F after eight weeks.
Photo board 2: Other wounds of patient 1 during the course of the treatment with maggot extracts. The photos show the outside of the right lower leg of the patient. Wound treatment of the female patient was done three times a week. After the maggot extract has been dissolved, it was dropped onto the wound. Treatment was done in the Clinic for Dermatology of the University Clinics Duesseldorf, Germany. Photo A shows wounds before the first treatment, photo B after two weeks of treatment, photo C after four weeks, and photo D after six weeks.
Case 2

Before treatment the 72-years-old female patient suffered from idiopathic ulcera crurum at both lower legs. The ulcers with a diameter of 10 cm each had been untreatable by the help of standard therapies. The patient additionally suffered from arterial hypertonia and chronic urticaria. Three months before treatment with maggot extracts it was tried to cover the ulcer with skin grafts.

Photo board 3: Wounds of patient 2 during the course of treatment with maggot extracts. The photos show the tibia and portions of the inside of the right lower leg. Wound treatment of the female patient was done three times a week. After the maggot extract has been dissolved, it was dropped onto the wound. Treatment was done in the Clinic for Dermatology of the University Clinics Duesseldorf, Germany. Photo A shows wounds four weeks before the first treatment, photo B directly before treatment, photo C after two weeks of treatment, photo D after four weeks, photo E after six weeks, and photo F after eight weeks.
Case 3

Before treatment the 74 aged female patient suffered from several ulcera crurum with different sizes at both lower legs for more than six months. Due to several persisting different diseases the origin of wounds was not clear. Since the patient refused to keep the wound dressing on the wound the patient was excluded from the study after two weeks of treatment.

Photo board 4: Wound of patient 3 during the course of treatment with maggot extract. The photos show outside of the left lower leg. Wound treatment of the female patient was done three times a week. After the maggot extract has been dissolved, it was dropped onto the wound. Treatment was done in the Clinic for Dermatology of the University Clinics Duesseldorf, Germany. Photo A was taken one week before the first treatment, photo B immediately before treatment, photo C after one week, and photo D after 2 weeks of treatment.
Case 4

The 85-year-old patient suffered from an ulcus cruris venosum at the right ankle. The patient additionally suffered from high blood pressure and multiple type IV sensitizations. After the first treatment the patient decided to switch to conventional maggot therapy.

Photo board 5: Wounds of patient 4 during the course of treatment with maggot extract. The photos show the outside of the left lower leg. Wound treatment of the male patient was done three times a week. After the maggot extract has been dissolved, it was dropped onto the wound. Treatment was done in the Clinic for Dermatology of the University Clinics Dusseldorf, Germany. Photo A shows the ulcer directly before treatment, photo B after one week of treatment with subsequent use of conventional maggot therapy.
Case 5

The 78-year-old male patient had a persistent extensive ulcus cruris mixtum at the left lower leg for about 18 months. The patient suffered from stage 4 peripheral artery occlusive diseases and had suffered several thromboses (e.g. in the arteria dorsalis pedis).

Photo board 6: Wounds of patient 5 during the course of treatment with maggot extract. The photos show the front view of the lower leg. Wound treatment of the male patient was done three times a week. After the maggot extract has been dissolved, it was dropped onto the wound. Treatment was done in the Clinic for Dermatology of the University Clinics Duesseldorf, Germany. Photo A shows the ulcus directly before the first treatment, photo B after two weeks, photo C after five weeks, photo D after seven weeks and photo E after eight weeks of treatment.
Photo board 7: Wound of patient 5 during the course of treatment with maggot extract. The photos show the inner surface of the left lower leg. Wound treatment of the male patient was done three times a week. After the maggot extract has been dissolved, it was dropped onto the wound. Treatment was done two times weekly by the patient himself and one time weekly in the Clinic for Dermatology of the University Clinics Duesseldorf, Germany. Photo A shows the ulcer directly before treatment, photo B after two weeks, photo C after five weeks, photo D after seven weeks and photo E after eight weeks of treatment.
Photo board 8: Wound of patient 5 during the course of treatment with maggot extract. The photos show outer surface of the lower left leg. After the maggot extract has been dissolved, it was dropped onto the wound. Treatment was done two times weekly by the patient and one time weekly by the in the Clinic for Dermatology of the University Clinics Duesseldorf, Germany. Photo A shows the ulcer directly before the first treatment, photo B after two weeks, photo C after five weeks, photo D after seven weeks and photo E after eight weeks of treatment.
Case 6

The 65-year-old male patient suffered from ulcera crurum on both sides of the lower legs for already several years.

Photo board 9: Wounds of patient 6 during the course of treatment with maggot extract. The photos show inner surface of the lower legs. Wound treatment of the male patient was done three times a week. After the maggot extract has been dissolved, it was dropped onto the wound. Treatment was done in the Clinic for Dermatology of the University Clinics Duesseldorf, Germany. Photo A shows wound on left lower leg and C wound on right lower leg directly before treatment, photo B shows wound on the left shaft after four weeks and photo D the wound on the right shaft after four weeks of treatment.
Case 7

The 61-year-old female patient suffered from local actinic keratosis. The ulcers developed quickly after excision of tissue samples. After 3 month of ulcer-persistence wound treatment with maggot extract was started. Relevant co-existing diseases were diabetes and high blood pressure.

Photo board 10: Wounds of patient 7 during the course of treatment with maggot extract. The photos show the left tibia and the inner surface of the left ankle. Wound treatment of the female patient was done three times a week. After the maggot extract has been dissolved, it was dropped onto the wound. Treatment was done in the Clinic for Dermatology of the University Clinics Duesseldorf, Germany. Photo A shows left tibia before the first treatment, photo C shows it after two weeks, photo E after four weeks, and photo G after seven weeks of treatment. Photo B shows the inner surface of the left ankle before treatment, photo D after two weeks, photo F after four weeks, and photo H after seven weeks of treatment.
Case 8

The 79-year-old female patient suffered from ulcera crurum venosa on both ankles for more than 3 years. There were several ulcers and sizes varied between 0.7 cm x 0.5 cm up to 14.5 cm x 5 cm. Analysis showed a chronic infection with methicillin resistant \textit{Staphylococcus aureus} (MRSA).

\textbf{Photo board 11:} Wounds of patient 8 during the course of treatment with maggot extract. The photos show both ankles. Wound treatment of the female patient was done three times a week. After the maggot extract has been dissolved, it was dropped onto the wound. Treatment was done in the Clinic for Dermatology of the University Clinics Duesseldorf, Germany. Photo \textbf{A} shows right ankle before treatment and photo \textbf{B} after seven weeks of treatment. Photo \textbf{C} shows left ankle before treatment and photo \textbf{D} after seven weeks of treatment.
Case 9

The 73-year-old male patient suffered from high blood pressure and ulcera crurum hypertonius (Martorell syndrome) on the left lower leg. One ulcer developed quickly after spindle biopsy and persisted for several weeks before start of the treatment with maggot extract.

Photo board 12: Wounds of patient 9 during the course of treatment with maggot extract. The photos show the right tibia and the right lower leg. Treatment was done two times weekly by the patient and one time weekly by the in the Clinic for Dermatology of the University Clinics Duesseldorf, Germany. Photo A shows the right tibia two weeks before, photo B the same wound directly before treatment, photo C after two weeks, photo D after four weeks, photo E after six weeks and photo F after eight weeks of treatment. Photo G and H show parallel but conventional treated ulcers on the right lower leg after six and eight weeks of treatment.
Case 10

The 72-year-old patient suffered from ulcera crurum varicosum close to both ankles for several years.

Photo board 13: Wounds of patient 10 during the course of treatment with maggot extract. The photos show both ankles of the patient. The photos show right tibia and the right lower leg. Treatment was done two times weekly by the patient and one time weekly in the Clinic for Dermatology of the University Clinics Duesseldorf, Germany. Photo A shows the left ankle before treatment, photo B after four weeks, photo C after five weeks, and photo D after eight weeks of treatment. Photo E shows medial view on the ankle before treatment, photo F after four weeks, photo G after five weeks, and photo H after eight weeks of treatment.
Results

Eight out of ten ulcers were colonized by *Pseudomoans aeruginosa* followed in number by colonization with *Staphylococcus aureus* (all sensitive for oxacillin) and *Proteus mirabilis*. Most notably *P. aeruginosa* showed phenotypes of intrinsic antibiotic resistances. One isolate was even resistant to three of four groups of antibiotics (3MRGN) except carbapenems. No evidence was obtained for an antibiotic mode of action of the extracts against any of the isolates. However considerable inhibitory effects of the extracts on the formation of biofilms by these isolates could be observed. Therapy of two patients was terminated by the treating physician due to bad compliance or multiple type IV sensitization that may have led to incompatibility. The experimental therapy of one patient did not show any effect. The treatment of all other patients led to better conditioning of the wound bed and/or to the formation of granulating tissue. Wounds of two patients showed complete re-epithelization after eight weeks of treatment. The analysis of wound exudates frequently showed dynamic changes in the concentrations of the different MMP in a weekly manner. However, a general suitability of any of these MMP as a marker could not be observed. Especially the often used concentrations of MMP-9 showed in the re-epithelialized wounds nearly constant concentrations during treatment. Concentrations of TIMP mostly showed dynamic changes during the treatment but decreased with increasing re-epithelization suggesting its general suitability as a marker. It is assumed, that the beneficial effects of the here studied extracts are based on the reduction of the ability for formation of biofilms by the wound colonizing bacteria thus making them more vulnerable to the host immune defense consequently leading to a recovery of the balance between MMP and TIMP. Investigations of effects of the here developed extract on primary human fibroblasts showed some effects on fibroblast vitality. However, grade of effects always ranged below a cytokine induced stimulus. Thus it is assumed that this effect may as well support the cutaneous wound healing.

References

