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Short communication

The natural medications for wound healing – Curcumin, Aloe-Vera and Ginger – do not induce a significant effect on the migration kinematics of cultured fibroblasts

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ABSTRACT

Curcumin, Aloe-vera and Ginger are popular natural medications (NMs) for treating wounds, however, the mechanisms by which these NMs apparently accelerate wound healing remain largely unknown. From a biomechanical perspective, it is specifically unclear whether fibroblast motility improves in the presence of any of these NMs. Here we use our recently developed quantitative high-precision automated assay for cell migration (Topman et al., 2012b) which is based on image processing of time lapse micrographs to determine whether kinematic parameters e.g. the maximum and average migration rates of *en mass* fibroblast colonies are influenced by treating the cells with the above NMs. We found no evidence that Curcumin, Aloe-vera and Ginger directly influence the *en mass* fibroblast migration kinematics *in vitro* post infliction of localized mechanical damage to the cultures. However, due to the complexity of a wound healing process *in vivo*, these NMs may still influence the healing through other pathways.

1. Introduction

In developed countries, natural medications (NMs) are widely used as non-prescription drugs for treating a spectrum of conditions. Conventional medicine generally takes a more conservative approach towards NMs (mostly due to lack of scientific evidence), but this does not appear to block use of NMs in mainstream complementary/alternative medicine, e.g. 42% of the U.S. population used NMs at least once. Greater numbers, 70% and 75% have been reported for Canada and France, respectively (WHO, 2002). In poor countries (or counties), NMs are also frequently being used given their availability and lower costs, e.g. up to 80% of Africans and 40% of Chinese use NMs (WHO, 2002). It therefore becomes increasingly important to scientifically characterize mechanisms by which NMs act.

Curcumin, Aloe-vera and Ginger are popular NMs for treating wounds. Curcumin, an active substance in the Turmeric shrub, was first used in Indian ayurvedic and traditional Chinese medicine. It is said to have anti-bacterial/viral/fungal/inflammatory qualities (Maheshwari et al., 2006). Aloe-vera, a gel produced from the leaves of a succulent plant, was known to the ancient Egyptian and Greek healers, and was used for wound healing by the armies of Alexander the Great and in the expeditions of Christopher Columbus (Surjushe et al., 2008). Aloe-vera, as well, is considered to have anti-inflammatory/viral and antiseptic effects (Surjushe et al., 2008). Ginger is produced from the rhizome of the *Zingiber officinale* plant, and was again used in Indian ayurvedic medicine, traditional Chinese medicine and by the ancient Greeks. It was also suggested to have anti-bacterial/inflammatory potency (Butt and Sultan, 2011).

There have been studies looking at the effectiveness of these substances in treating wounds inflicted to animal models. It was found that abrasions in rats treated with corticosteroids heal faster when also treated with Curcumin, Ginger or combination of both (Bhagavathula et al., 2009), and that laser-induced or irradiation wounds in mice heal better by topically treating them with Curcumin (López-Jornet et al., 2011; Jagetia and Rajanikant, 2012). Oral administration of Aloe-vera was also found to be effective in improving healing of irradiation wounds in mice (Atiba et al., 2011). However, the mechanisms by which these NMs apparently accelerate wound healing remain largely unknown. From a biomechanical perspective, it is specifically

Abbreviations: AMR, average migration rate; MMR, maximum migration rate; NM, natural medication; TEMCM, time for end of mass cell migration; TOMCM, time for onset of mass cell migration

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unclear whether fibroblast motility improves in the presence of any of these NMs. Since this question would be rather difficult to address in animal studies, we use our recently developed quantitative high-precision automated assay for cell migration (Topman et al., 2012a,b) to determine here whether the *kinematics* of *en mass* fibroblast migration is influenced by treating the cells with either Curcumin, Aloe-vera or Ginger.

2. Methods

2.1. Cells and materials

NIH3T3 fibroblasts (ATCC) were cultured in growth medium composed of Dulbecco's modified Eagle's medium, 10% fetal bovine serum, 2 mM Glutamine and a 0.1% antibiotic cocktail (Biological Industries, Israel). Cells were first thawed from N_2 storage into 25 cm^2 flasks and kept in an incubator at 37 °C, 5% CO_2. When reaching near-confluency, cells were subcultured into 75 cm^2 flasks by removing the medium, washing twice with PBS, applying Trypsin–EDTA (0.25%/0.25%) and incubating for 5 min. Thereafter, cells were subcultured at a 1:10 ratio into 75 cm^2 flasks and passaged every 3–4 days. Three days prior to damage infliction, cells were seeded in aliquots of 250×10^3 into 6-well plates and cultured up to confluency.

Curcumin was purchased (C1386, Sigma) whereas Aloe-vera (Alfa-lax, Tivon biotech, Israel) and Ginger (*Zingiber officinale*, Solgar) were produced from capsule contents of non-prescription nutritional supplements (available in pharmacies). None of these NMs is soluble in water. Hence, each was dissolved in a suitable solvent using the lowest possible solvent concentration (Table 1), after ensuring that solvent concentrations were non-toxic. Curcumin and Aloe-vera were dissolved in dimethyl sulfoxide (11 mg:ml) and Ginger was dissolved in ethanol (1.6 mg:ml).

2.2. Experimental design

Localized culture damage was inflicted by a metallic micro-indentor (diameter ~420 μm) which consistently created geometrically well-defined areas denuded of cells, termed herein as 'wound' (Fig. 1). The medium was replaced immediately following damage infliction to remove debris. Wound micrographs (resolution 2560×1920; 3 pixels/μm) were captured automatically every 2 h for ~14 h at the same field-of-view, using a digital camera (DS-Fi1, Nikon) connected to a phase-contrast microscope (Eclipse TS100, Nikon).

Kinematic parameters of the *en mass* migration post damage infliction (Fig. 1) were determined using a previously reported method (Topman et al., 2012a,b) which is summarized here for completeness: An image-processing-based algorithm (Fig. 2) continuously segments between denuded and cell-populated areas by calculating spatial and temporal local texture homogeneity measures, which then generates wound-area-versus-time plots that are fitted with Richards-type functions. The Richards functions are used to derive the following outcome measures which describe the *en mass* migration kinematics (Fig. 2): (i) maximum migration rate (MMR), (ii) time for onset of mass cell migration (TOMCM)=when 10% of the wound area is covered, (iii) time for end of mass cell migration (TEMCM)=when 95% of that area is covered, (iv) average migration rate (AMR)=the linear slope between the wound areas at the TOMCM and TEMCM time points, and (v) the integral of the area-curve over time.

Curcumin, Aloe-vera and Ginger were tested 6 times each (in different cultures) with respect to their own controls which contained only the solvent in the medium (Table 1). Cell viability was verified in all trials and non-damaged regions remained fully viable and confluent (in presence of the solvents) throughout the experimental periods.[2]

One-way analysis of variance (ANOVA) was used (per each outcome measure) to determine first whether there are differences between controls associated with each NM type, and since none emerged, all control data were pooled together to increase the statistical power. A second one-way ANOVA followed by Tukey–Kramer comparisons was then used to identify potential differences between the pooled controls and the 3 NM conditions. A p-value <0.05 was considered significant.

3. Results

Cells migrated from across the entire perimeter of the damage sites following the pattern reported previously (Topman et al., 2012a,b) (Fig. 1). The TOMCM measures were the only ones to show significant differences, between Curcumin and Ginger, but each was statistically indistinguishable from the control data (Fig. 3). All other measures of the NM groups were indistinguishable from controls (Fig. 3). Hence, we conclude that none of the NMs tested herein influenced the migration kinematics of fibroblasts.

Table 1
Concentrations of natural medications (NMs) and corresponding solvents. DMSO=dimethyl sulfoxide.

NM	Curcumin	Aloe-vera	Ginger
Solvent	DMSO	DMSO	Ethanol
NM concentration (μg/ml)	7.3	29.3	5.3
Solvent concentration (μl/ml)	0.66	2.6	3.3

In preliminary studies we tested lower concentrations of these NMs, but did not find a statistically significant and consistent effect (either stimulatory or inhibitory) on the *en mass* migration kinematics with respect to controls, and so have increased the doses of each NM up to its solubility level as above.

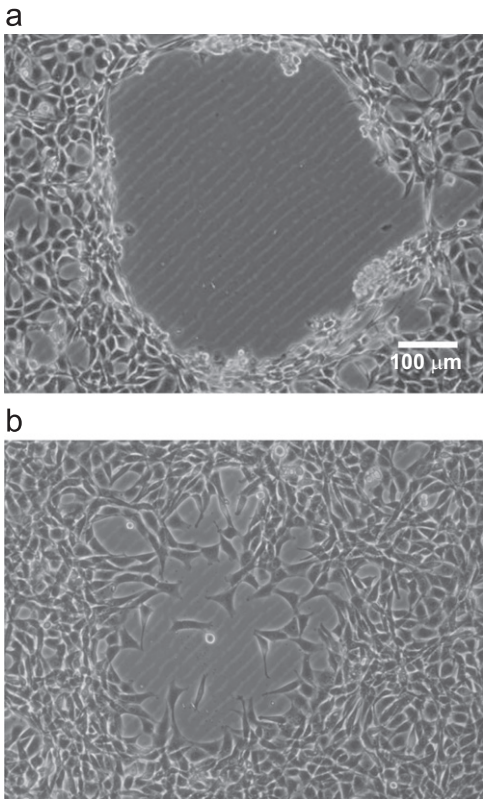

Fig. 1. Example micrographs of a 'wounded' fibroblast monolayer: (a) immediately post infliction of the damage and (b) 12 h afterwards.

4. Discussion

We used a cellular biomechanics approach where automated quantitative wound healing assays did not identify significant effects of Curcumin, Aloe-vera and Ginger on *en mass* migration kinematics of fibroblasts in damaged cultures. The negative findings in this regard are highly valuable for scientifically characterizing the mechanisms and pathways by which these NMs potentially act—which is a matter of serious debate but also where solid scientific evidence is poor.

[2] We used lactic acid (L1875, Sigma) with 55 mM HEPES as positive control, which significantly decreased the MMR and AMR of fibroblast colonies to half their control levels, and nearly doubled the TEMCM (Topman et al., 2012a).