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Cytotoxicity testing of silver-containing burn treatments using primary and immortal skin cells

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ARTICLE INFO

Article history:
Accepted 12 February 2014

Keywords:
Cytotoxicity
Hydrogel
Dressing
Silver nanoparticles
Burn care

ABSTRACT

A novel burn wound hydrogel dressing has been previously developed which is composed of 2-acrylamido-2-methylpropane sulfonic acid sodium salt with silver nanoparticles (silver AMPS). This study compared the cytotoxicity of this dressing to the commercially available silver products; Acticoat™, PolyMem Silver® and Flamazine™ cream. Human keratinocytes (HaCaT and primary HEK) and normal human fibroblasts (NHF) were exposed to dressings incubated on Nunc™ polycarbonate inserts for 24, 48 and 72 h. Four different cytotoxicity assays were performed including; Trypan Blue cell count, MTT, Celltiter-Blue™ and Toluidine Blue surface area assays. The results were expressed as relative cell viability compared to an untreated control. The cytotoxic effects of Acticoat™ and Flamazine™ cream were dependent on exposure time and cell type. After 24 h exposure, Acticoat™ and Flamazine™ cream were toxic to all tested cell lines. Surprisingly, HaCaTs treated with Acticoat™ and Flamazine™ had an improved ability to survive at 48 and 72 h while HEKs and NHFs had no improvement in survival with any treatment. The novel silver hydrogel and PolyMem Silver® showed low cytotoxicity to all tested cell lines at every time interval and these results support the possibility of using the novel silver hydrogel as a burn wound dressing. Researchers who rely on HaCaT cells as an accurate keratinocyte model should be aware that they can respond differently to primary skin cells.

1. Introduction

The increase of antibiotic resistance in infected wounds has lead to the need to develop more agents that can be used to treat colonized wounds effectively. There is substantial evidence to support the use of silver containing products in infected wound management and silver has been used for infection treatment for centuries [1]. More recently, silver sulphadiazine (e.g. Flamazine™) is commonly used to treat burn wounds [2]. In the last decade, a number of silver products have been introduced, which are available in different formulations and contain various forms of silver including: pure metallic silver and compounds such as silver

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http://dx.doi.org/10.1016/j.burns.2014.02.009
0305-4179/

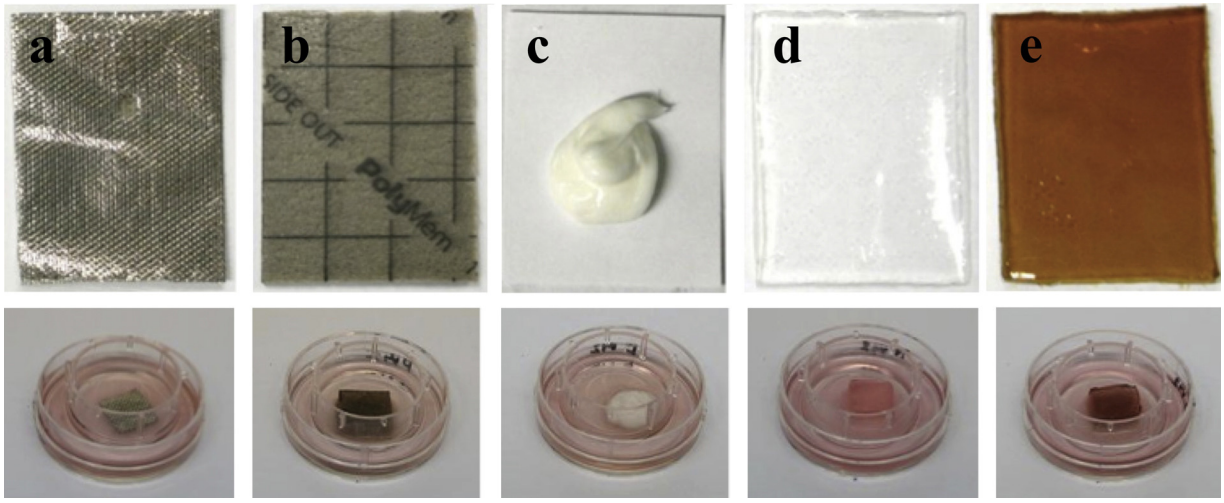

Fig. 1 – Photos of burn products tested in this study (top) and of the Nunc™ polycarbonate inserts with dressings or cream on top incubated with HaCaT cell cultures for 24 h (below); (a) Acticoat™, (b) PolyMem Silver®, (c) Flamazine™ cream, (d) neat hydrogel and (e) silver hydrogel.

phosphate, silver sulfadiazine, silver-sodium carboxymethyl cellulose and silver chloride [3]. Recent advancements in nano-technology have lead to the development of nanocrystalline silver, and a new dressing coated with silver nanoparticles (SNPs) for burn treatment (Acticoat™) [4].

Various research groups have studied the cytotoxicity of silver products using different cell lines and various cytotoxicity assays. In 2004, a cytotoxicity study using MTT assays to assess the effect of nanocrystalline silver dressing (Acticoat™) on primary human keratinocytes proposed that Acticoat™ was not appropriate for use as a topical dressing for cultured skin grafts [5]. Another study used MTT assays on primary human keratinocytes and fibroblasts and found Acticoat™ was likely to produce significant cytotoxic effects on both cell lines, whereas PolyMem Silver® showed the least toxicity compared to other silver-based dressings tested [6]. Our research group previously found that Silvazine™ (which has ceased production) and its replacement Flamazine™ cream, had cytotoxic effects on HaCaT cells demonstrated by a Toluidine Blue staining assay [7]. PolyMem Silver® was found to have low toxicity on HaCaT cells assessed by counting surviving cells after incubation with treatments [8].

Recently, a dressing containing SNPs has been developed by our research group [9], which is composed of 2-acrylamido-2-methylpropane sulfonic acid (AMPS) sodium salt hydrogel. The hydrogel acts to provide a moist environment to stimulate healing, while absorbing wound exudate during the healing process. It feels cool to touch, which may reduce the pain of wounds. The transparency of the hydrogel enables observation of the wound healing process. Silver has been incorporated into the dressing to help prevent wound infection. Although hydrogels have been used previously on burns to keep them moist and silver-containing products have also been used in burn care, this novel treatment combines both advantages in the one dressing. It is also relatively economical to produce. The antibacterial activity of the novel silver dressing against Methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* has been evaluated using bactericidal measurement (broth culture and plate count method) [9] and the results support the possibility of using 5 mM silver Hydrogel as an antimicrobial burn wound dressing.

In this study, the cytotoxicity of the novel silver hydrogel dressing (containing 5 mM silver) was compared to the commercially available silver products: Acticoat™, PolyMem Silver® and Flamazine™ cream, with neat AMPS hydrogel (containing no silver) used as a negative control. Three cell monolayer culture systems were compared: HaCaT (a human keratinocyte immortalized cell line), HEK (primary human epidermal keratinocytes) and NHF (primary normal human fibroblasts), to investigate the cytotoxicity of the silver agents.

2. Methods

2.1. Cytotoxicity assessment

2.1.1. Burn wound products

Three common silver-containing burn treatments were used in this experiment as a comparison for the silver hydrogel dressing: Acticoat™, PolyMem Silver® and Flamazine™ cream (Fig. 1 and Table 1). The neat hydrogel (containing no silver) served as a negative control.

2.1.2. Cell culture systems

HaCaT cells were a gift from Dr N. Fusenig (German Cancer Research Centre, Heidelberg, Germany) [10]. The primary keratinocytes and fibroblasts were obtained from foreskin surgical discards obtained with institutional ethics approval. Both keratinocyte cell lines were cultured on 35 cm diameter tissue culture plates at a seeding density of 5000 cells/cm^2 in 2 ml of growth medium. HaCaTs grew in Roswell Park Memorial Institute (RPMI) media (Gibco, Australia) containing