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Letter to editor

Antimicrobial potential of a gel containing hydrogen peroxide and hyaluronic acid

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Dear Editor,

Although biocidal action of hydrogen peroxide (HPO) is not well understood, many authors believe that the free hydroxyl radicals causes oxidation of DNA, proteins and lipid membranes (Linley et al., 2012). Use of HPO on skin problems is recently gaining importance due to its efficacy in preventing acne (Milani et al., 2003; Veraldi et al., 2016). It is also being used in treating Molluscum contagiosum due to its antiviral properties (Schianchi et al., 2018). Hyaluronic acid (HA) is another product with promising skin benefits because of its moisturising and wound healing properties, along with its ability to prevent wrinkles (Lee et al., 2015). It also produces extracellular matrix and prevents infections by modulating cellular immunity (Jegasothy et al., 2014). There are also clinical studies that tested the effectiveness of HA on skin, particularly in decreasing the depth of wrinkles, hydrating the skin and improving the firmness and elasticity of the skin (Jegasothy et al., 2014; Lee et al., 2015; Pavicic et al., 2011).

The sensitivity of hyaluronan polysaccharidic chain to radical degradation promoted by HPO decomposition, alone or in presence of metal ions like iron and copper is well known. Nevertheless, combination of these two molecules have been used to develop therapeutical approaches against tumour growth, thanks to the viscosity of HA in water solution, which facilitates slower rate of degradation of HPO *in vivo* (Abbasi et al., 2021; Akima et al., 2016). As a consequence, oxygen delivery occurs for longer time and locally, improving efficacy and allowing to use lower dosage of HPO, less than the common 3% used in antiseptic application. Such lower concentrations of HPO undoubtedly have advantages in terms of cell viability of the tissues treated and reduction in polysaccharide degradation. Therefore, a formulation combining both these products was developed and tested on common skin bacteria and *Candida albicans*.

To our knowledge, we could not find any research that tested the effectiveness of this combination through *in vitro* or *in vivo* studies. However, a combination of HPO with other agents, such as retinoids, has been tested. A Randomised Clinical Trial testing a combination of HPO and adapalene found it to have a comparable effectiveness on skin acne and more tolerability than benzoyl peroxide combined with adapalene (Capizzi et al., 2004). We tested the effectiveness of the novel combination of HPO and HA as gel (Ialuxid, BMG Pharma company, Italy; composition: aqua, hydrogen peroxide, carbomer, xanthan gum, hydroxyethyl acrylate / sodium acryloydimethyl taurate copolymer, squalane, glycine, sodium hyaluronate, polysorbate 60, oxyquinoline sulfate) in comparison to neomycin and a control group (glycerol) on several microbial strains including *Cutibacterium acnes, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Streptococcus Gordonii, Pseudomonas aeruginosa, Klebsiella pneumoniae and*

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TABLE 1: Growth conditions and Inoculum concentration of each microbial strain used in this analysis.

STRAIN	Growth conditions	Inoculum concentration (CFU/plate)
Pseudomonas aeruginosa ATCC 9027	Casein Soya Bean Digest Agar (3-4 days – 37°C)	2.5×10^3
Staphylococcus aureus ATCC 6538	Casein Soya Bean Digest Agar (3-4 days – 37°C)	6.0×10^3
Staphylococcus epidermidis ATCC 12228	Casein Soya Bean Digest Agar (3-4 days – 37°C)	3.5×10^3
Streptococcus pyogenes ATCC 12344	Casein Soya Bean Digest Agar (5-6 days – 37°C – Anaerobiosis)	4.5×10^3
Streptococcus gordonii ATCC 10558	Casein Soya Bean Digest Agar (3-4 days – 37°C – microaerohilic)	3.7×10^4
Klebsiella pneumoniae ATCC 4352	Casein Soya Bean Digest Agar (3-4 days – 37°C)	7.4×10^3
Propionibacterium acnes ATCC 6919	Casein Soya Bean Digest Agar (3-4 days – 37°C)	6.8×10^4
Candida albicans ATCC 10231	Sabouraud-dextrose agar (3-4 days – 25°C)	4.5×10^2

TABLE 2: Antimicrobial effectiveness of the diluted and undiluted test and control products on the common skin microbes.

		HPO+HA		Neomycin		Control
		Undiluted	1:10	Undiluted	1:10	
Cutibacterium acnes	Microbial growth reduction	100%	100%	100%	100%	0
	Inhibition halo	20.5mm	12mm	2.5mm	4mm	0mm
Staphylococcus aureus	Microbial growth reduction	100%	100%	100%	100%	0
	Inhibition halo	18.5mm	8.5mm	5mm	5.5mm	0mm
Staphylococcus epidermidis	Microbial growth reduction	100%	95.2%	99.5%	38.8%	0
	Inhibition halo	25mm	0.5mm	0.5mm	0mm	0mm
Streptococcus pyogenes	Microbial growth reduction	100%	100%	100%	100%	0
	Inhibition halo	13.5mm	7mm	5.5mm	5mm	0mm
Streptococci Gordonii	Microbial growth reduction	100%	100%	6.15%	1.04%	0
	Inhibition halo	8mm	4mm	0mm	0mm	0mm
Pseudomonas aeruginosa	Microbial growth reduction	100%	100%	100%	100%	0
	Inhibition halo	10.5mm	6.5mm	2.5mm	4mm	0mm
Klebsiella pneumoniae	Microbial growth reduction	100%	100%	100%	100%	0
	Inhibition halo	11mm	5mm	1.5mm	3.5mm	0mm
Candida albicans	Microbial growth reduction	100%	6.06%			0
	Inhibition halo	1.5mm	0mm			0mm

Candida albicans. As this was an *in vitro* experiment, no ethics approval was required. The *C. acnes* is the most common bacteria associated with surgical site contamination and resides in the deep dermal layer of the skin within pilosebaceous glands and hair follicle. Surgeons rely on antibiotics in an attempt to eradicate these bacteria preoperatively – antibiotics have been the treatment of choice, while HPO has recently been found to show promising results in preventing these bacteria (Hernandez et al., 2019). Although *S. aureus* and *S. epidermidis* are common pathogens on the skin, they are involved in opportunistic infections, particularly in patients with burns (Gallagher et al., 2007). The *S. Pyogenes* is also a common group A streptococcus that colonises nasopharynx, skin and oral cavity while *S. gordonii* is mostly found in the oral cavity (Nobbs et al., 2007). Candida species are common skin pathogens and *C. albicans* is most often associated with symptomatic skin infections (Kühbacher et al., 2017), interactions between candida and streptococci are also not uncommon (Koo et al., 2018). In addition, *P. aeruginosa,* which causes varying levels of skin and soft tissue infections, (Bassetti et al., 2018) and *K. pneumoniae,* less commonly reported in skin infections, were also considered (Chang et al., 2008).

To investigate antimicrobial activity, an inhibition contact test was conducted on solid-agar cultures. For this purpose, 25mm cellulose discs were used as support for contact of the sample with the microbial cultures. Antimicrobial activity of the tests and control products was evaluated through the observation of the microbial growth reduction below the application area and by measuring the inhibition halo around it. The original lyophilized strain was grown in a suitable liquid growth medium and plated on solid medium by isolating individual colonies which were then amplified and frozen at -30°C in aliquots of known titre. Preparation of the strains for the test was carried out starting from an aliquot thawed, taken up in liquid medium, isolated and then allowed to grow by incubating for specific conditions. Each strain was inoculated into a layer of agar surface at a concentration indicated in Table 1; the concentration of viable cells was determined by the method of the plate count.

Undiluted and diluted (1:10) product was placed on 25mm cellulose supports until completely adsorbed and the supports were then placed on each of the inoculated medium plates. Medium plates not inoculated and untreated were used to check sterility of the culture medium. The experiment was repeated when the medium plates couldn't be evaluated. Effect of HPO+HA, neomycin and control was tested on all strains except *C. albicans,* which was not tested with neomycin.

HPO+HA had excellent antimicrobial effect against all the bacterial strains, both when tested undiluted and diluted. However, its effect on *C. albicans* was only observed when undiluted (Table 2). Neomycin was also found to have excellent antimicrobial effect against *P. aeruginosa, S. aureus, S. pyogenes, K. pneumoniae, C. acnes*, both when tested undiluted and diluted while its effectiveness on *S. epidermidis* was only observed when tested undiluted, with a minimal antimicrobial effect when tested diluted. Neomycin did not present any antimicrobial effect against *S. gordonii*. Results from our preliminary *in vitro* research indicates that the new combination has promising role in skin infections due to its effectiveness on several common skin microbes. We are intending to conduct clinical studies to further establish its effectiveness.

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