The Modulating Action of Silver Nanoparticles on Collagen Deposition in Producing Scarless Wound Healing

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ABSTRACT INTRODUCTION:
Healing of burn wound is a complex process that is often addressed to problems of undesired cosmesis and mechanical property in the regenerated skin. Silver nanoparticles (AgNPs) are known to be bactericidal\(^1\). Its application to wound results in accelerated healing and excellent cosmesis with regeneration of hair follicles\(^2\). However, the skin’s mechanical property which indicates the functionality of skin is yet to be understood. Since it is known that collagen is the major source of strength and elasticity in skin\(^3\), the aim of this study was to study the effect of AgNPs on collagen formation and deposition at complete wound healing as it was hypothesized that AgNPs could improve mechanical property of the repaired skin due to its ability to modulate collagen deposition. Tensile test was performed to study the skin modulus, with collagen staining and SEM imaging to reveal the collagen expression and characteristics.

METHODS:
Skin defects were created on mice by excision of their full thickness dorsal skins, which was approved by the CULATR 1974-09, The University of Hong Kong. The wounds were topically applied with AgNPs or left untreated. The regenerated skins were harvested for evaluation. Three experimental groups were set up: normal (n=7), AgNPs-treated group (n=5) and untreated (n=5). Macroscopic and microscopic appearance was compared. Tensile tests were performed to evaluate the skin modulus and results were statistically analyzed by Student’s paired t-test (p<0.05). Masson Trichrome and immunohistochemical staining were employed to investigate collagen type I and III expression. Quantitative collagen content detection was compared. Tensile tests were performed to study the collagen architecture of the skins of different groups.

RESULTS SECTION:

The macroscopic and microscopic appearance of AgNPs treated skin resembled closely with normal skin with hair follicles regenerated but not in the untreated ones. The tensile modulus for normal, AgNPs and untreated group were 4.7±1.33 MPa, 4.9±1.73 MPa and 1.2±0.8 MPa respectively. No statistical significance was found between normal and AgNPs group (p=0.85) whereas the untreated group was found to be significantly different to both normal (p=0.0004) and AgNPs group (p=0.0026). Immunostaining showed higher collagen intensity in control and AgNPs than untreated group. Medugorac’s collagen detection suggested a protein to collagen ratio of 0.71, 0.64 and 0.33 for normal, AgNPs and untreated sample respectively. SEM illustrated well-oriented collagen fibrils in normal and AgNPs group while exhibited random chaotic alignment in untreated sample. The average fibril diameters (n=100) measured were 104.3±19.0 nm, 78.6±13.2 nm and 64.8±11.5 nm for control, AgNPs and untreated samples respectively.

Fig.1 Collagen fibres were stained in blue with Masson Trichrome (A-C). Expression of collagen type I (D-F) and III (G-I) were blotted with immunohistochemical staining. The normal and AgNPs group showed similar colour intensity and distribution of collagen protein, while untreated group exhibited considerably lighter colour and observance of cryptogenic structure. Scale bar: 20 µm.

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REFERENCES:

DISCUSSION:
Wound healing with AgNPs demonstrated scarless healing with mechanical property resembling the normal skin. Skin modulus was found to correlate to the level of collagen content, its physical characteristics as well as the organization\(^1\). With observation of higher collagen density, better spatial distribution, increased fibril size and well-aligned fibrils in normal and AgNPs group, the higher skin modulus measured can be explained. However, this study only allowed the surface collagen fibrils and matrix to be characterized.

The overall improvement in appearance and mechanical property were attributed to the ability of AgNPs in modulating collagen deposition, resulted in excellent fibril morphology and alignment. This is believed to be achieved through a regulated differentiation of fibroblast by AgNPs\(^3\) that inhibited uncontrolled growth of collagen, which is a problem often occurred in natural healing process. Yet, the molecular pathway for which AgNPs affects collagen regeneration would still need further investigation.

To conclude, this study has given us an insight into the effect of AgNPs in restoring strength in skin during the healing process and have led us one step towards the clinical application of AgNPs in wound treatment.