THE SUSTAINABLE RELEASE OF VANCOMYCIN FROM MICRO- AND NANOSTRUCTURED COLLAGEN LAYERS

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[ENGINEERING OF BIOMATERIALS 138 (2016) 40]

Introduction

The infection of implanted endoprostheses represents a serious problem as far as orthopaedic and trauma surgery are concerned. One of the ways in which to increase the efficacy of the therapy is to use a local antibiotic delivery system [1]. Local delivery of antibiotics maximizes target tissue concentration, and minimizes systemic toxicity risks. Technology and conditions during such carrier's preparation are very important aspects as they can greatly affect the final release profile of antibiotics or their transformation to microbiologically inactive products. The aim of the study was to compare biodegradable composite layers prepared by different techniques. They should release active form of Vancomycin in a sustained and controlled manner effectively for 3 weeks at concentrations exceeding minimum inhibitory concentration for vancomycin-resistant *Staphylococcus aureus* (VRSA) (>16 mg/L) without initial burst releasing.

Materials and Methods

Micro- or nanostructured layers were prepared based on collagen (type I, VUP Medical, CZ), 0, 5 or 15 wt% of hydroxyapatite nanoparticles (avg. 150nm, Sigma Aldrich) and VANCO (Vancomycin hydrochloride, Mylan S.A.S, France) in amount of 10 wt% of total weight of collagen (COL) with hydroxyapatite (HA). Micro-structured layers were prepared by means of lyophilisation of COL/HA/VANCO dispersion [1]. Nano-structured layers were prepared employing the electrospinning (4SPIN, Contipro, CZ) of 8wt% collagen solution with dispersed HA particles. VANCO was applied by two different procedures, i.e. directly to COL solution before electrospinning or subsequent impregnation of electrospun COL/HA cross-linked layers, respectively. The stability of all collagen layers were enhanced by cross-linking with EDC/NHS (Sigma Aldrich) [1]. The solid phase extraction method and HPLC analysis [2] (Agilent 1200, diode array detectoreDAD, Agilent Tech.) were used to characterize the *in vitro* release rates of VANCO and its crystalline degradation antibiotically inactive products over a 21-day period (PBS, 37°C). The antimicrobial effects of the layers were determined using agar diffusion testing against VRSA isolates.

Results and Discussion

The structure of micro and nanostructured layers is illustrated at FIG. 1. The maximum concentration of the released active form of vancomycin (approx. 700 mg/l after 3 hours, 150 mg/l 21st day) was assessed by means of the vancomycin impregnation of cross-linked electrospun layers.

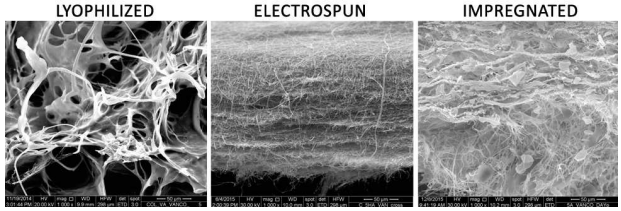

FIG. 1. Representative SEM images of COL/5%HA/ VANCO layers prepared by different techniques (x1000).

The lowest concentration was determined for those layers electrospun directly from a COL solution with VANCO (see FIG. 2). Modification using hydroxyapatite exerts no strong effect on vancomycin evolution.

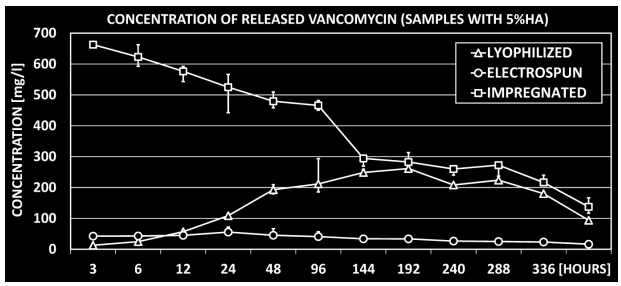

FIG. 2. An example of the concentration of released active form of vancomycin from layers with 5%HA (median, interquartile range).

Agar diffusion tests (FIG. 3) revealed that the electrospun impregnated layers exhibited the highest activity. All the tested layers showed release of an active form of VANCO release at concentration higher than MIC.

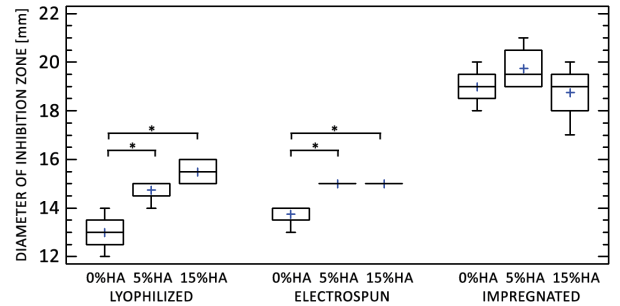

FIG. 3. Diameter of the inhibition zone against *S. aureus* as function of different HA concentrations and different preparation techniques (Mann-Whitney, 0.05).

The higher specific surface of nanostructured layers probably plays a negative role in the preparation process due to the higher rate of VANCO elution to the cross-linking solution. This may be overcome via the subsequent impregnation of the cross-linked layers.

Conclusions

Our results suggest that the local application of high-dose vancomycin via drug delivery carriers provides a safe therapeutic osteomyelitis treatment method that prevents the development of bacterial resistance.

Acknowledgments

This study was supported by a grant provided by the Technology Agency of the Czech Republic under project no. TA04010330.

References

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