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[1] Department of Oral and Maxillofacial Surgery, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
[2] Spintec Engineering GmbH, Aachen, Germany
[3] BLS Laboratories GmbH, Berlin, Germany
[4] Department of Oral- and Cranio-Maxillofacial Surgery, Klinikum rechts der Isar der Technischen Universität München, Munich, Germany
[5] Department of Experimental Orofacial Medicine, Philipps University Marburg, Marburg, Germany
[6] Institute of Textile Machinery and High Performance Material Technology, Technische Universität Dresden, German

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Smeets R.[1], Knabe C.[5], Rheinnecker M.[2], Gröbe A.[1], Heiland M.[1], Zehbe R.[3], Kolk A.[4], Sachse M.[5], Große-Siestrup C.[3], Wöltje M.[6], Hanken H.[1]

Novel silk protein barrier membranes for guided bone regeneration

Introduction

Different types of bioresorbable and nonresorbable membranes have been widely used for guided tissue regeneration (GTR). An alternative could be the use of silk-membranes (Fig. 1) which exhibit several advantages. During manufacturing individual modifications are possible, no infection risks are associated with their implantation and the mechanical characteristics are excellent [1-8]. In this study we examined the binding of hydroxyapatite (HA) and beta-Tricalcium phosphate (ß-TCP) to silk-membranes and evaluated the effects on cell proliferation in vitro and effects on facilitating bone formation and defect repair during guided bone regeneration.

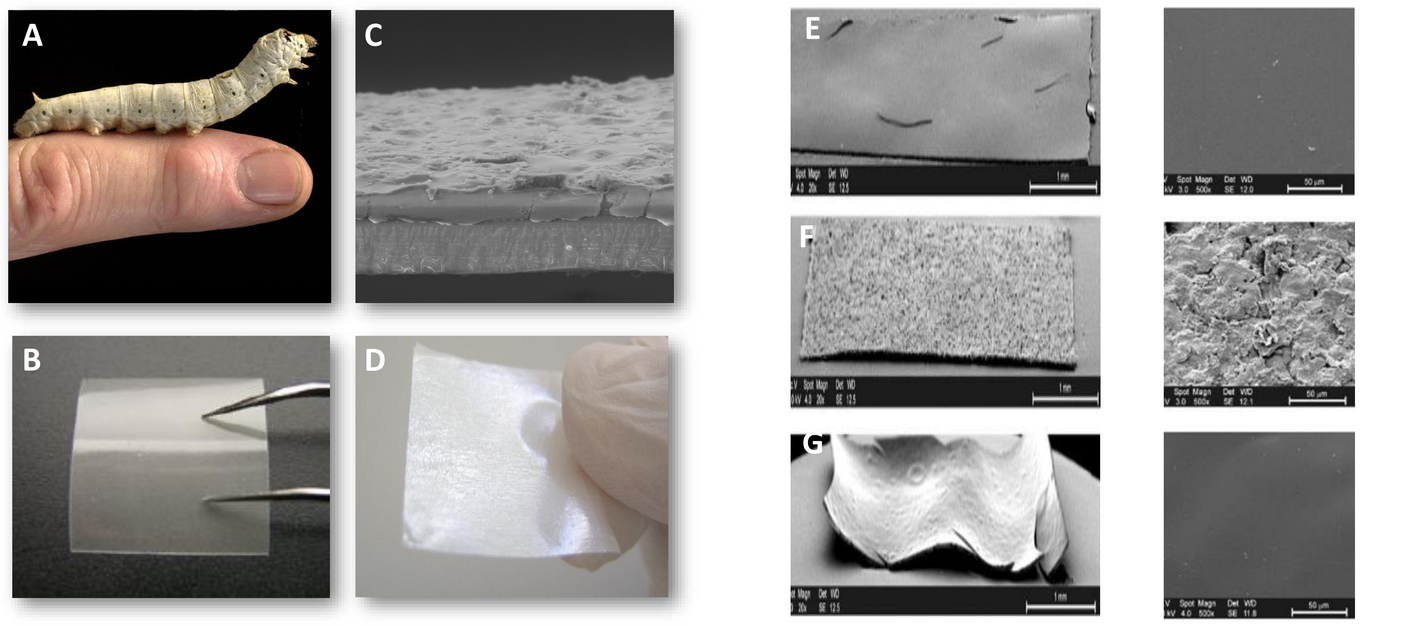

Fig. 1: *Bombyx mori* silk worm (A); unmodified silk membrane (B); scanning electron micrographs of an unmodified silk membrane (D, E), a hydroxyapatite-modified silk membrane (F) and a ß-TCP modified silk membrane (C, G) at two different magnifications. Magnification: 20x (left) and 500x (right).

Material and Methods

Two calvarian bone defects of 12 mm (Fig. 2) in diameter were created in each of a total of 38 rabbits and four different types of membranes, (silk-, hydroxyapatite-modified silk-, β-TCP-modified silk- and conventional collagen) were implanted to cover one of the two defects in each animal. Hematology, body weight and general health were monitored throughout the 10 weeks of the study period which were all within the normal range for all animals and histologic analysis did not show any adverse reactions in any of the defect sites, demonstrating good biocompatibility of all silk protein membranes.

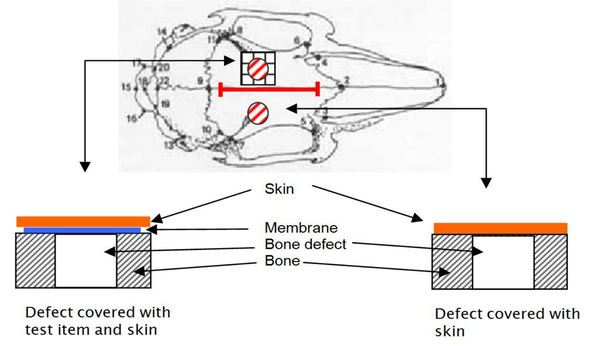

Fig. 2: Schematic illustration of the two surgically created calvarial bone defects in each rabbit. One of the two defects (in the illustrated case, the left one), was covered with a silk-barrier membrane prior to repositioning of the full thickness skin flap and subsequent wound closure. With the other cranial defect wound closure was achieved by directly repositioning the full thickness skin flap. This defect served as a control.

Results

After 10 weeks, the collagen membrane was resorbed in all cases, while the silk membrane was still visible in 1/5 (20%) and the hydroxyapatite-silk membrane in 4/5 (80%) cases in the micro-CT scans. β-TCP-modified silk membranes remained visible in all cases. Histomorphometric evaluation revealed significantly higher (p=0.002) new bone ingrowth into defects covered with β-TCP modified silk membranes compared to new bone ingrowth into defects without any barrier membrane cover. The highest rate of new bone ingrowth was observed in defects protected with β-TCP silk membranes (Fig. 3,4).

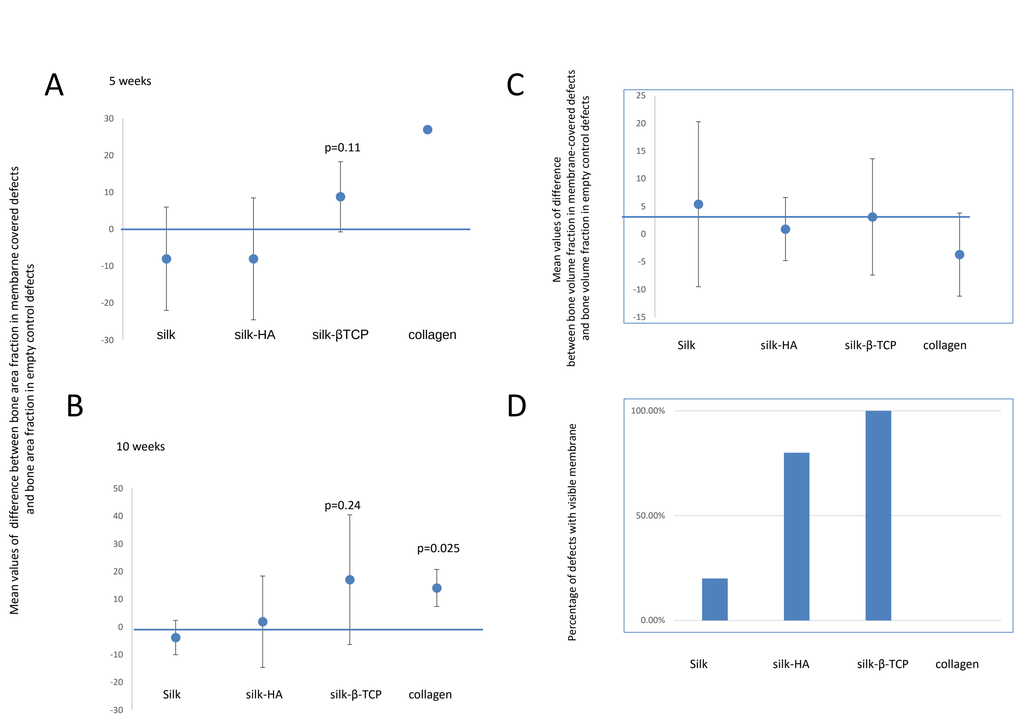

Fig. 3: Diagram depicting the results of the histomorphometric evaluation of bone formation and ingrowth into the defect 5 **(A)** and 10 **(B)** weeks after surgery (SIS Analysis™ software). Diagrams depicting the results of the micro-CT analysis. **(C)** Visualizes the mean values of the difference between bone volume fraction (bone volume / total defect volume) in membrane vs. without membrane - protected defects. **(D)** Shows the percentage of defects with visible membrane. A significant difference (p=0.003) was noted between groups regarding percentage of defect sites in which membranes were present without resorption.

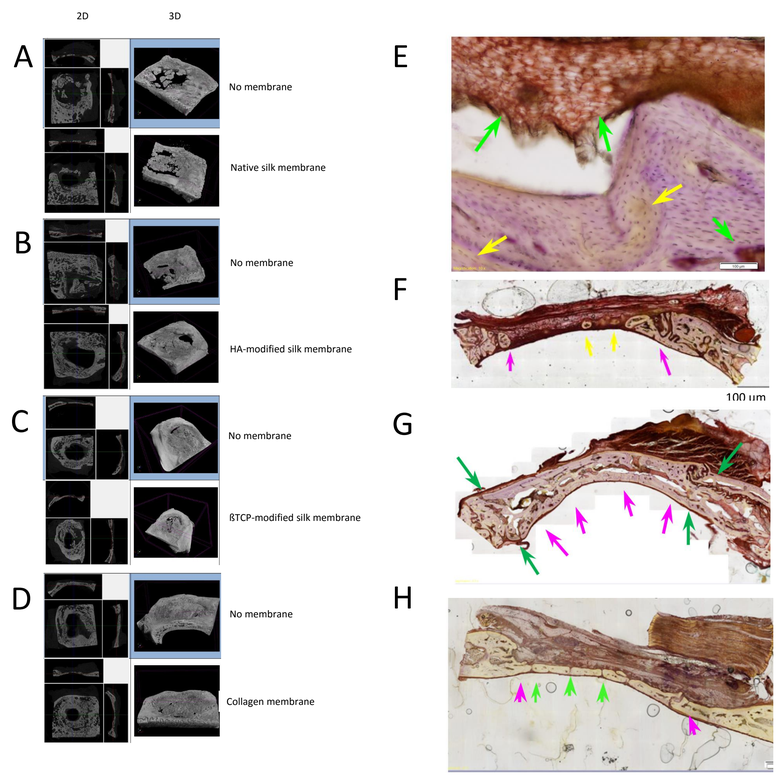

Fig. 4: Micro-CT scans of the defect area obtained in the same animal 10 weeks after the surgery; 2D scans **(left column)**, 3D visualization of the volumetric data **(right column)**. Defects without barrier membranes **(upper images)** and membrane-covered defects **(lower images)**, utilized with: native silk membrane **(A)**, a HA-modified silk membrane **(B)**, a β-TCP-modified silk membrane **(C)**, and collagen membrane **(D)**.
(E): Strong staining for type 1 collagen and alkaline phosphatase in bone marrow space **(green arrows)**, weak staining for alkaline phosphatase in the mineralized bone matrix **(yellow arrows)** shows active bone healing
(F): Histomicrograph control: cross section of an empty cranial defect 10 weeks after surgery. Only beginning woven bone formation and ingrowth into the defect at the periphery **(pink arrows).** Small areas of beginning woven bone formation in more central defect areas **(yellow arrows)**. Immunodetection of alkaline phosphatase, undecalcified sawed section counterstained with hematoxylin. Scale bar = 100 µm.
(G): Histomicrograph: cross section of a membrane protected cranial defect with TCP-modified silk membrane (10 weeks post placement). Almost complete restoration of the original calvarial bone morphology and microarchitecture **(pink arrows)** showing two cortical layers. Original defect margins **(green arrows)**. Deacrylized sawed section immunostained for type I collagen and counterstained with hematoxylin. Scale bar = 2 mm.
(H): Histomicrograph: cross section of a membrane protected cranial defect with collagen membrane (10 weeks post placement). Progressing bone formation and almost complete defect bridging from the defect margins **(pink arrows)** towards the defect center; remaining soft tissue **(green arrows)** shows less mature bone restoration (only one cortical layer compared to two with the β-TCP-modified silk membrane).

Conclusion

The highest rate of new bone ingrowth was observed in defects protected with β-TCP silk membranes. No other membrane showed a comparable effect on guided bone regeneration with respect to promoting significantly greater bone regeneration and defect bridging.

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Contact: Ralf Smeets, M.D., D.M.D., Ph.D.
Department of Oral- and Maxillofacial Surgery, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
Phone.: +49 - 40 – 741054001 Fax: +49 - 40- 741055467
E-mail: r.smeets@uke.de