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Review Article

Increased Adhesion of (Cna) Positive *Staphylococcus Aureus* to Damaged Articular Cartilage

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Abbreviations: CFU: Colony Forming Units; *Can:* Collagen-Adhesin Gene; *Cna*(+): *Cna*-Positive; *Cna*(–): *Cna*-Negative; ECM: Extracellular Matrix; FITC: Fluorescein Isothiocyanate; LFD: Large Field Detector; MSCRAMM: Microbial Surface Components Recognizing Adhesive Matrix Molecules; PBS: Phosphate-Buffered Saline; *S.: Staphylococcus;* SEM: Scanning Electron Microscope; TSB: Trypticase Soy Broth

Background

Septic Arthritis As Well As Acute Or Chronic Osteomyelitis Caused By Hematogenous Spread Or Direct Inoculation In Open Trauma Or Surgical Intervention Affect Hundreds Of Thousands Patients Each Year. *S.Aureus* Is One Of The Main Pathogens Associated With This Disease [1-3], Possessing A High Variance Of Virulence Mechanisms Promoting Attachment, Immune Evasion, Release Of Exotoxins, And Biofilm Formation [4,5]. Once Walled Off *In Vivo*, Biofilms Can Disseminate Disease In A Host By Detachment And Subsequent Planktonic Migration To New Infection Sites [6,7]. After The Attachment, The Efficient Regulatory Network Of *S.Aureus* Quickly Activates Virulence And Evasion Factors In Response To Changing Environmental Conditions. *S.Aureus* Can Change Its Lifestyle Between Adherent And Aggressive Forms In Response To Bacterial Density [8]. From A Patho-Physiological Point Of View, The Initial Binding Of Pathogens To Surfaces Is Most Important For Preventing Further Disease. *S.Aureus* Is Able To Bind Specific Host Matrix Proteins Via Distinct Bacterial Cell Surface Receptors (Adhesins) [9,10]. Different *S.Aureus*-Strains Show Different Patterns When Binding To Various Extracellular Matrix Proteins Mediated By So-Called "Microbial Surface Components Recognizing Adhesive Matrix Molecules" (Mscramms). These Adhesins Also Mediate Adhesion To Prostheses By Binding To Host Proteins That Cover Implant Surfaces *In Vivo* [11]. Gene-Encoding *S.Aureus*-Mscramms Have Been Identified That Bind Fibronectin, Fibrinogen, Vitronectin, Thrombospondin, Elastin, Osteopontin, And Collagen [12-20]. The Role Of The Gene-Encoding Collagen-Adhesin *Cna* Is Highlighted By The Observation That The Mutation Of This Gene Eliminates The Ability Of The *S.Aureus*-Phillips Strain To Bind To Collagen [18]. Furthermore, This Mutation Can Be Ideally Used In Control Patterns Regarding Bacterial Adhesion To Collagen-Rich Surfaces, Such As Bone And Cartilage, Which Are Preferential Sites Of Staphylococcal Infection [18]. Collagen-Binding Adhesins Seem To Be Important In The Pathogenesis Of Osteomyelitis And Septic Arthritis [21].

Articular Cartilages Have A Distinct Architecture With A Specific Distribution Of Collagen And Glycosaminoglycan Content As Well As A Collagen Type From The Surface Down To The Tight Mark. Their Most Superficial Layer ─ Termed "Lamina Splendens" ─ Has A Maximal Thickness Of 200 µm, Is Totally Cellular, And Consists Of A Dense Collagen Network, Mainly Collagen Type-I [22]. Specific Anti-Adhesive Glycoproteins, Such As Superficial Zone Proteins, Can Be Found In This Area [23,24]. Beyond The Lamina Splendens Lays An Area Rich In Collagen And Fibronectin Containing Several Chondrocytes. The Subsequent Deeper Cartilage Zones Are Characterized By High Amounts Of Collagen Type-II And Glycosaminoglycan-Containing Proteoglycans [25]. It Is Yet Unknown If Degenerative Or Traumatic Surface Characteristics Of Articular Cartilages Influence Bacterial Adhesion. Therefore, We Investigated The Adhesion Capacity Of *S.Aureus* To Different Articular Cartilage Surface Conditions. The Following Conditions Were Defined: Lamina Splendens, Cut Surfaces Of The Middle Zone And Degraded Lamina Splendens, And Degraded Cut Surfaces. It Is

Of Interest If The Binding Capacity Of *S.Aureus* To Collagen May Be Affected By Damaged Cartilage Surface Structures Exposing Collagen Fibers. For That Reason, Bacterial Strains Were Chosen With An On/Off Effect In Collagen-Binding Capacities, I.E., *S.Aureus Cna*-Positive Strains (*Cna* (+)) And *Cna*-Negative Strains (*Cna* (–)).

Materials And Methods

Bacterial Strains

S.Aureus ATCC 25923, Proven To Adhere To Collagen *In Vitro*, Was Used As A *Cna*(+) Strain [26] And *S.Aureus* LS-1, Which Does Not Express A Collagen-Adhesin, Served As A *Cna*(–) Strain. *S.Aureus* LS-1 Was Originally Isolated From A Swollen Joint Of A Spontaneously Arthritic Mouse Kindly Provided By Ing-Marie Jonsson, Department Of Rheumatology And Inflammation Research, Gothenburg, Sweden [27].

Bacterial Adherence To Extracellular Matrix Molecules

To Test The Specific Binding To Extracellular Matrix Molecules Of Both *S.Aureus* Strains Used In The Present Study, We Used A Recently Published Dot-Blot-Array Technique [28]. Nitrocellulose Membranes (Invitrogen, Carlsbad, CA) Were Spotted With 1 µl Volumes Containing Human Serum Albumin, Fibronectin, Gelatin, And Type-I Collagen. We Started With A Concentration Of 1 Mg/Ml Followed By A Two-Fold Concentration Dilution Series. The Membranes Were Overlaid With Fluorescein Isothiocyanate (FITC)-Labeled *Cna*(+) *S.Aureus* 25923 And *Cna*(–) *S.Aureus* LS1 For 1 H At Room Temperature. After Having Been Washed With A Buffer Solution, The Fluorescence Of Protein-Bound Bacteria Was Detected With A Typhoon 9200 Imaging System (Amersham Biosciences, Freiburg, Germany), In Which Dark Spots Are Indicative Of Strong Binding Activities (Figure 1).

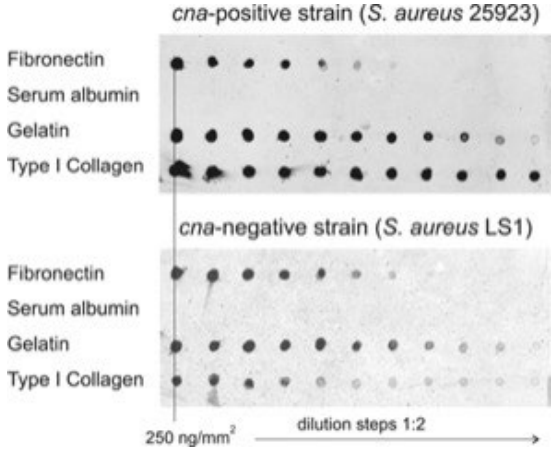

Figure 1: The Binding Properties Of The Bacterial Strains Employed In This Study To Proteins Of The Extracellular Matrix. Gelatin And Human Serum Albumin Served As Controls. Proteins Were Immobilized As Dots In Serial Dilutions On Nitrocellulose Membranes. The Binding Of Fluorescence-Labeled Bacteria To Immobilized Proteins Was Measured By A Fluorescence Scanner And Is Visualized By Dark Spots.

Articular Cartilage Preparation

Articular Cartilages Were Harvested From 12 To 16 Week-Old Calves From The Tibial Plateau. According To The Guidelines For Experimental Research On Animals, We Used Animal Tissue From Calves Purchased From A Local Butcher. The Ethics Committee Of The University Regensburg Stated That No Approval Was Necessary. The Hind Legs ─ Truncated In One Piece To Keep The Knees Sterile ─ Were Dissected Under Sterile Conditions Into The Femoral And Tibial Part. From The Medial And Lateral Tibial Plateau, We Cut Osteochondral Blocks Of 1 Cm² And A Depth Of 2 Cm With A Reciprocal Saw. After Securing The Blocks To A Sledged Microtome (HM 440 Micron), We Produced 500 µm Thick Slides That Were Further Trimmed With A Circular Knife (Diameter 6 Mm), Which Resulted In Defined Cylindrical Probes (Figure 2). The Articular Cartilage Probes From The First Cut Carrying The Lamina Splendens Comprised Group A. A Second Cut Of The Same Size Was Made From The Middle Zone Originating From A Depth Of 500 To 1000 µm Of The Articular Cartilages. These Specimens With Different Surface And Biochemical Characteristics Comprised Group C. The Specimens Were Kept Moistened All The Time With Phosphate-Buffered Saline (PBS, Ph 7.4) Without Any Supplements. The Vitality Of The Cartilage Discs Was Investigated With The LIVE/DEAD® Cell-Mediated Cytotoxicity Kit (L-7010, Molecular Probes Eugene, Oregon, USA). The Discs Were Separately Held In 24 Well-Plates In PBS. Disk Samples With And Without Lamina Splendens Were Trypsinated With A Concentration Of 0.05 µg/Ml Under Gentle Nutating Conditions At 37°C For 30 Min. After The Degradation Process, The Discs Were Rinsed In PBS With 0.05 µg/Ml Anti-Trypsin For 30 Min And Three Times With PBS. The Degraded Articular Cartilage Blocks Containing Either Lamina Splendens Or Originating From The Middle Zone Of The Articular Cartilages Comprised Sample Groups B And D.

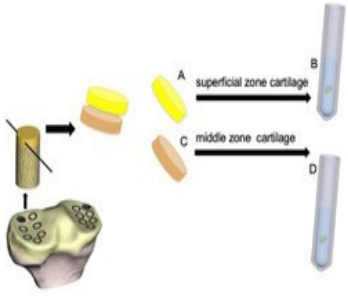

Figure 2: Preparation Of Bovine Articular Cartilage Explants. Osteochondral Fragments Were Harvested From The Tibial Plateau Using A Punch Measuring 4 Mm In Diameter. Two Slices Measuring 0.5 Mm In Thickness Were Cut From The Top Of The Osteochondral Fragments. The First Harvested Cartilage Disc Contained The Superficial Joint Surface, I.E. The Lamina Splendens (A). The Second Harvested Disc Only Represented Cut Surfaces From The Middle Zone (C). Parts Of These Samples Were Further Degraded By Trypsin-Treatment Resulting In Degraded Superficial (B) And Degraded Middle Zone Surfaces Of Articular Cartilages (D).

Cartilage Inoculation With Bacterial Strains

Bacteria Were Cultured On Columbia Blood Agar Plates Over Night At 37°C. The Plates Were Then Kept At 4°C. Before The Experiments, Staphylococcal Strains Were Streaked For Isolation On A Fresh Columbia Agar Plate And Incubated Overnight At 37°C. A Few Colonies From The Plate Were Inoculated In 10 Ml Of Trypticase Soy Broth (TSB) And Incubated With Gentle Shaking (250 Rpm) At 37°C For 24 H. Bacteria Were Harvested By Centrifugation At 4000 Rpm For 10 Min And Washed Three Times With 5 Ml Sterile PBS For 5 Min. Afterwards, Bacteria Were Re-Suspended In Sterile PBS With A Concentration Of About 10^8 Colony-Forming Units (CFU) Per Ml (OD600 Of 0.9). Single Cartilage Discs Were Incubated In 1 Ml Bacterial Suspension At Room Temperature With Gentle Shaking (150 Rpm) For 3 H. After This Incubation Period, The Discs Were Washed Three Times With 1 Ml Sterile PBS To Remove Unbound Bacteria From The Discs.

Release And Determination Of Adhering Bacteria

Attached Staphylococci Were Eluted From The Disc Surfaces With 1 Ml Sterile PBS Per Disc By 10 Min Sonication (Bandelinsonorex RK 100 H, Berlin, Germany; 160 W, 1 A, 220-240 V, 35 Khz) On Ice. 100 µl Of Each Eluate In PBS Was Inoculated In Dilution Series Onto Columbia Blood Agar Plates And Incubated Overnight At 37°C. We Counted The Number Of Colony-Forming Units Of *S.Aureus* Eluted In 100 µl On The Agar Plates And Calculated The Total Number Of Staphylococci Per Disc.

Fluorescence Microscopy

To Show That *S.Aureus* Was Accurately Eluted From The Disc Surfaces, We Compared The Bacterial Density On The Disc Surfaces Before And After Sonication By Fluorescence Microscopy (Figure 3). Discs Were Stained With A Two-Color Fluorescence Assay (LIVE/DEAD^{Ó} BaclightÔ Bacterial Viability Kit For Microscopy L-7007, Molecular Probes, Eugene, Oregon, USA) By 15 Min Incubation In The Dark, Examined Under A Fluorescence Microscope (Leitz DM RBE), And Finally Photographed. This Test Was Done In Duplicate For Each Group.

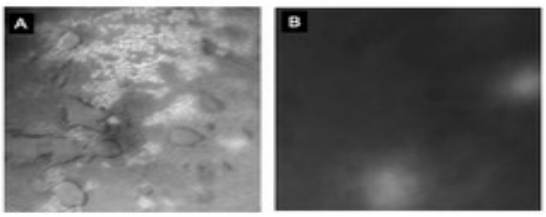

Figure 3: Fluorescence Microscopy Analysis Was Used For Measuring The Effectiveness Of Cartilage Disc Sonication To Release Adherent Bacteria. Before The Sonication Process, Numerous Fluorescent Bacterial Colonies (*Cna* (+) Strain) Were Visible On Degraded Superficial Cartilage Discs (A). After The Sonication Process, No Fluorescent Bacterial Colonies Were Visible (B).

Scanning Electron Microscopy (SEM)

Articular Cartilage Surface Structures Were Analyzed With An FEI Quanta 400F (Eindhoven, Netherlands) Environmental Scanning Electron Microscope (ESEM). Adhesion Of *Cna*(+) And *Cna*(–) *S.Aureus*-Strains Was Visualized On All Surface Conditions By SEM Using The Low Vacuum Mode At A Pressure Of 0.83 Torr At Room Temperature. Cartilage Specimens Were Mounted Onto Stubs, Placed Directly Into The SEM Chamber Without Drying Or Fixation, And Tilted By 30 Degrees For Analysis. For Bacteria Imaging, We Used A 30-µm Incident Beam Aperture With A Spot Size Of 4 Nm, An Accelerating Voltage Of 4 Kv, And A Large Field Detector (LFD).

Statistical Analysis

We Analyzed The Bacteria Colonies On The Various Articular Cartilage Surfaces In Quadruplicate (4 Specimens) In Each Experiment, Which Was Repeated Three Times (N=16). Differences Between Median Values Of Bacteria Numbers On The Various Cartilage Surfaces Were Statistically Analyzed With The Mann-Whitney-U-Test (SPSS/PC+, Version 16.0G SPSS, Chicago, IL, USA) For Pair-Wise Comparisons Among Groups At A $P<0.05$ Level Of Significance.

Results

Bacterial Adherence To Extracellular Matrix Molecules

As Shown In Figure 1, No Bacterial Binding To Serum Albumin Could Be Observed For Both Strains, Whereas Binding To Other ECM Proteins Strongly Depended On The Amount Of Immobilized Protein And The Bacterial Strain Examined. Both Strains Bound To Fibronectin And Gelatin In A Dose-Dependent Manner Without Any Substantial Differences Between The Two Strains. However, Collagen Type-I Mediated Strong Binding Only For The *Cna*(+) Strain *S.Aureus* 25923, Whereas Binding Of The *Cna*(–) Strain *S.Aureus* LS1 Was Substantially Weaker But Not Completely Inhibited.

Effect Of Sonication On Bacterial Release From Articular Cartilages

Sonication Is Known To Release Adhered Bacteria From Solid Surfaces, Such As Implants, Even Those Covered In A Biofilm. In Our Experiments, The Density Of *S.Aureus* On The Different Cartilage Disc Surfaces Was Detected By Eluting The Bacteria In PBS Via Sonication And Consecutive Counting Of Cfus. The Efficiency Of The Removal Process Was Characterized By Fluorescence Microscopy To Detect Any Remaining Bacterial Cells On The Articular Cartilage Surface. Figure 3A Illustrates That *Cna*(+) Bacteria Can Be Detected Before The Sonication Process But Not Afterwards (Figure 3B). No Photo Taken Showed Any Residual Bacterial Colony After Sonication. Thus, This Procedure May Represent A Valid Method For Detecting Living Bacteria,

Such As *S.Aureus,* Bound To Cartilage Surfaces. Furthermore, Both Bacterial Strains Were Directly Suspended To The Columbia Blood Agar Plates In A Concentration Of 10^8 Colony-Forming Units (CFU) Per Ml (OD600 Of 0.9) To Compare The Growth Behavior Of *Cna*(+) And *Cna*(–) Strains. No Difference In CFU Was Observed Between The Two Strains. Therefore, Different Growing Behavior Of The Two Bacteria Strains On Columbia Blood Agar Plates Can Be Excluded.

Effects Of Bacterial Strain Selection And Cartilage Surface Condition On Bacterial Adherence

When Comparing Both *S.Aureus*-Strains Regarding Their Adhesion To Cartilage Surfaces We Found Higher Numbers Of *S.Aureus* If The Factor "*Cna"* Was Present (Fig. 4, P<0.001). Furthermore, For The *Cna*(+) Strain, The Affinity To Bind To Cartilage Surfaces Was Strongly Influenced By The Factor "Cartilage Surface Condition". Higher Numbers Of Bacteria Were Found On Surfaces Prepared From The Middle Zone Than From The Upper Zone Cartilage (Block C Vs. Block A). Adhesion Of *Cna*(+) *S.Aureus* Was Remarkably Increased On Surfaces Degraded By Trypsin-Digestion In Contrast To Non-Degraded Surfaces Of The Upper Zone (P<0.05, Block A Vs. Block B). The Highest Rate Of Bacterial Adhesion Was Found On Degraded Surfaces From The Middle Zone Cartilage (Figure 4, Block D). Adhesion Of The *Cna*(–) Strain To Different Cartilage Surfaces Was Low And Did Not Vary Significantly.

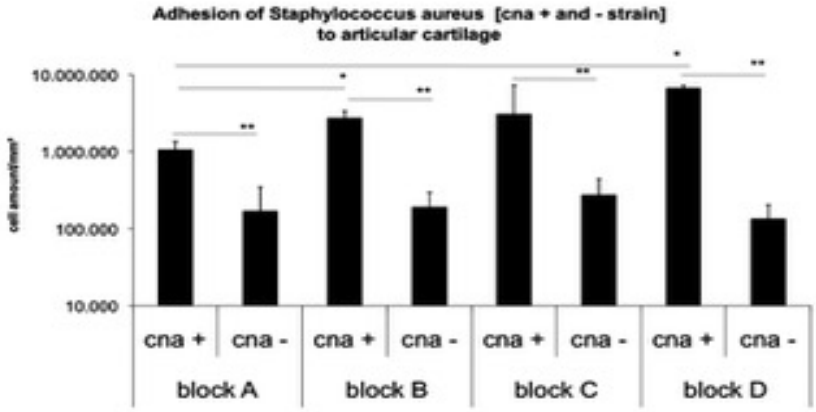

Figure 4: Bacterial Colonies Released From Colonized Articular Cartilage Disks And Cultured On Columbia Blood Agar. Significantly (**, P<0.001), Cna(–) *S.Aureus* Adhered To Cartilage Discs In Smaller Amounts Than Cna(+) Strains. Furthermore, Degraded Superficial And Middle-Zone Surfaces Were More Vulnerable To Cna(+) *S.Aureus*-Colonization Than Untreated Surfaces (*, P<0.05).

Scanning Electron Microscopy (SEM) Evaluation Of Bacterial Strains Adhered To Articular Cartilages

SEM Analysis Clearly Showed That Different Pre-Treatments Of Cartilage Discs Resulted In Different Surface Structures (Figure 5 and 6). Cuts From The Middle Zone Had The Smoothest Surface Followed By Intact Lamina Splendens (Figure. 5A, 5C, 6A, and 6C). Enzymatic Disk Degradation Led To A Release of The Extracellular Matrix, Exposure Of The Collagen Network, And Uncovered Collagen Fibrils On Both Surface Patterns (Figure 5B, 5D, 6B, and 6D). Bacterial Colonization Depending On The Cartilage Surface Structure Can Be Visualized By SEM. Remarkably, SEM Images Showed More Colonies Of The *Cna*(+) Strain Attached To Degraded Cartilage Surfaces And Even Attached Single Bacterial Cells Bound To Exposed Collagen Fibers (Figure 5D). SEM Pictures Of Cartilage Discs After Incubation With The *Cna*(+) *S.Aureus*-Strain Always Show Bacterial Colonies (Figure 5). The Healthy Surface Of The Lamina Splendens Was Rarely Covered By *Cna*(+) *S.Aureus*-Cells (Figure 5A) In Contrast To The Surface Of Degraded Lamina Splendens Showing Pronounced Colonization Of This Strain (Figure 5B). *Cna*(+) *S.Aureus*-Cells Also Attached In High Numbers To The Cut Surface Of The Middle Zone Even Without Enzymatic Surface Degradation (Figure 5C). After Additional Enzymatic Digestion, Single Bacterial Cells Were Visible That Were Directly Bound To Surface-Exposed Collagen Fibers (Figure 5D).

Pictures Of Healthy (Figure 6A) And Degraded Lamina Splendens (Figure 6B) After Incubation With A *Cna*(–) *S.Aureus*-Strain Showed Only Few Bacterial Colonies On Both Surface Conditions. For This Strain, Hardly Any Colonization Was Visible On The Cut Surface Of The Middle-Zone Cartilage (Figure 6C), Even After Surface Degradation By Trypsin-Digestion (Figure 6D).

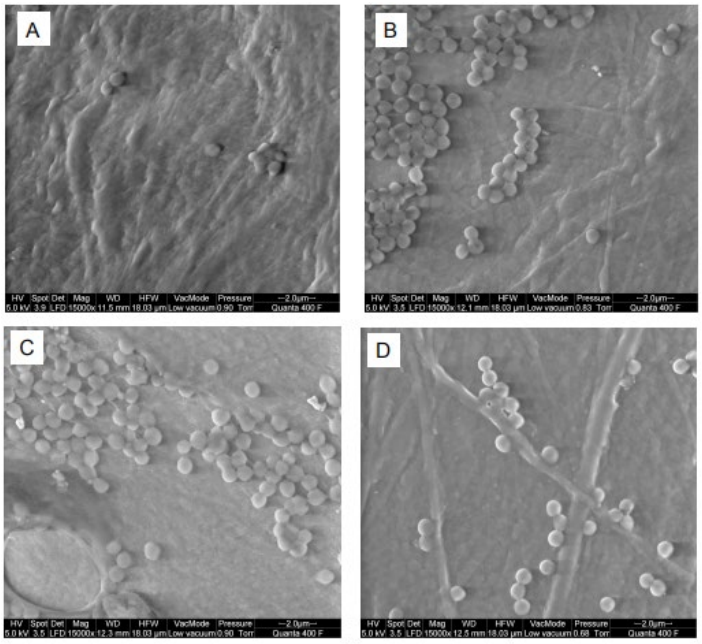

Figure 5: SEM Analysis Of Cartilage Surfaces Inoculated With The *S.Aureus* Cna(+)-Strain. The Intact Lamina Splendens Shows Very Few Attached Bacterial Cells (A), Whereas High Amounts Of Cells Are Visible After Trypsin-Treatment (B). On The Middle-Zone Articular Cartilage, A Large Amount Of Cna(+) *S.Aureus*-Bacteria Was Found On Both Non-Degraded (C) And Trypsin-Treated Surfaces (D). After Surface Degradation With Trypsin, Adhesion Of Single Bacterial Cells To Exposed Collagen Fibers Becomes Visible (D).

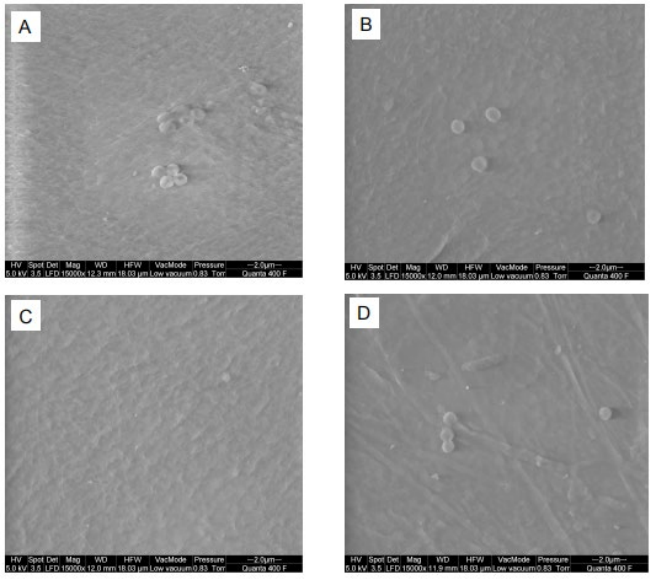

Figure 6: SEM Analysisi of Cartilage Surfaces Inoculated With The *S.Aureus* Cna(–)-Strain. Low Bacterial Adhesion Is Visible On Intact (A) And Degraded Lamina Splendens Surfaces (B) As Well As On Non-Degraded (C) And Trypsin-Treated (D) Surfaces Of Middle-Zone Cartilage Blocks.

Discussion

Sonication And Bacterial Adhesion Assay

Implants With Clinical And Radiological Signs Of Loosening Are Often Classified As Being Sterile Because Of Negative Microbiological Testing. Trampuz, et al. Showed Significant Improvement In The Detection Of Bacterial Implant Loosening By Sonication Of Removed Implants [29]. In His Study, Patients With Aseptic Joint Prosthesis Loosening Were Further Investigated By Sonication Of Their Explanted Prosthesis And By Microbiological Testing. Adherent Bacteria Were Reported In Up To 70% Of Patients. We Used Sonication To Release Attached Bacteria From A Biological Material, i.e., Articular Cartilage. Because This Tissue Can Absorb Sonication Waves, Adherent Bacteria May Not Be Released In The Same Manner As From Metal Implants. Therefore, Preliminary Tests With Fluorescence Microscopy Showed That The Tested *S.Aureus*-Strains Could Also Be Released By This Method. As Far As We Know, This Method Has Not Yet Been Described For Application With Articular Cartilages.

Comparable Studies And Clinical Relevance

Previous Studies Have Shown That The Expression Of The Collagen-Adhesin *Cna* Is Necessary And Sufficient To Mediate Attachment Of *S.Aureus* To Articular Cartilages [26,30]. *Cna* Has Been Described As A Virulence Determinant In Experimental Septic Arthritis [18], Showing A Direct Correlation Between The Affinity For Collagen And The Virulence Of *S.Aureus*-Strains [21]. The Collagen-Adhesion Factor *Cna* Does Not Seem To Be The Only Critical Factor, And Its Expression Is Not Enough To Turn Bacterial Hosts Into Pathogens [31]. Additionally, According To Elasri, et al. *Cna* Does Not Cause Joint Infections But Contributes To The Ability Of *S.Aureus* To Induce Osteomyelitis, Because No Difference Was Found Between Septic Arthritis In Mice Infected With A *Cna*(+) *S.Aureus*-Strain Or Its *Cna*(–) Mutant [32]. In Our Experiments, We Could Also Not Detect Any Remarkable Difference Between *Cna*(+) And *Cna*(–) *S.Aureus*-Strains Regarding The Adhesion Of *S.Aureus* To Normal Articular Cartilages. However, Collagen-Binding Seems To Be A Critical Factor In Arthritis Because Of The Destruction Of Articular Cartilage Surfaces. The Fact That Bacterial Binding To Damaged Or Degraded Articular Cartilages Is Significantly Higher Than To Intact Cartilages Confirms The Hypothesis That Destroyed Lamina Splendens Favors The Adhesion Of And Infection With *Cna*(+) *S.Aureus*-Strains. In Animal Models, *Cna*(–) Isogenic Mutants Were Significantly Less Virulent Than Parental Strains, So That Collagen-Adhesion *Cna* Also Presents An Attractive Target For Developing Novel Vaccines [18].

Molecular-Biological Aspects Of Collagen-Adhesin *Cna*

Articular Cartilage Surfaces Are Diminished Even In Early Stage Osteoarthritis. Bacterial Arthritis As Well As Acute And Chronic Osteomyelitis Caused By Hematogenous Spread Or Direct Inoculation In Open Trauma Or Surgical Intervention Affect Hundreds Of Thousands Of Patients Each Year. These Disabling Conditions Are Notoriously Resistant To Host Defenses, Surgery, And Antibiotic Treatment. *S.Aureus* Is The Most Frequent Organism Associated With Bacterial Arthritis And Osteomyelitis, Causing Up To 80% Of Cases. *S.Aureus* Is Able To Bind Specific Host Matrix Proteins [9]. Degenerated Or Injured Articular Cartilages May Be Another Virulent Factor For The Development Of Septic Arthritis. Degraded Lamina Splendens, Native Articular Cartilage Surfaces In Contrast To Degraded Deeper Articular Cartilage Blocks Results In Different Released Molecules And Exposed Extracellular Matrix Structures (Fig. 5A Vs. Fig. 5B). The Lamina Splendens Is Characterized By Special Components, Such As The Superficial Zone Protein (PRG4) [33,34]. This Molecule Has Lubricating Abilities And Presumably Binds To Fibronectin And Collagen [35]. Potentially, This Mechanism Could Also Be Used For Inhibiting *S.Aureus*-Adhesion To Intact Lamina Splendens When Developing An Anti-Adhesin For Bacteria.

This Study Presents A New Model For Investigating Bacterial Adhesion To Tissue As A Main Virulence Factor. The Adhesion Of *S.Aureus* To Articular Cartilages Depends On Its Capacity To Bind To Collagen Mediated By Receptor Expression (*Cna*), Which Can Be Determined As A Virulence Factor For Bacterial Arthritis And Osteomyelitis. Therapeutic Interventions May Block The Corresponding Epitopes On Collagen Structures For The *Cna*-Receptors Of Bacteria. Healthy Cartilage Surfaces Containing The Lamina Splendens With Its Lubricating Glycoproteins Show A Natural Barrier To The Adhesion Of *Cna*(+) *S.Aureus*-Strains. Besides A Sterile Work Place, Healthy Articular Cartilage Surfaces Seem To Be The Best Barrier To Prevent Post-Operative Empyema, Even Against Bacterial Strains Capable Of Expressing The *Cna*-Gene. For Surgeons, The Best Defense Against Post-Operative Infections Is Protecting Articular Cartilage Surfaces In Arthroscopic Or Open Joint Surgery.

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