Effect of Wake Extension and Short Recovery Sleep on Objective Vigilance and Subjective Sleepiness in Young Adolescents

2023 Lifespan Research Day Abstract Submission Contest

Research Category: Basic Science

Primary Research Location: E.P. Bradley Hospital Sleep Research Laboratory

Funded By: K01MH109854; P20GM139743

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Abstract

Background: Adolescents routinely experience insufficient sleep. Compared to adults, less is known regarding how wake extension and recovery sleep impact objective and subjective indices of vigilance in youth. Here we explore these questions using acute wake extension in the laboratory.

Methods: Twenty–two 10–13–year–old children (12.03±1.14 years, 12F) ranging in parent–rated inattention (Conners–3–Parent t=38–76) completed a week of actigraphy–monitored at–home sleep stabilization (9.5h TIB). Participants then slept in the laboratory with polysomnography for a baseline (BSL) opportunity of 9.5h (21:00–06:30) and returned the subsequent evening for wake extension (WE) to 02:30 and a 4h recovery sleep opportunity (02:30–06:30). Participants completed batteries including a 5–minute tablet–based psychomotor vigilance task (PVT; BrainBaseline) and subjective state ratings at 20:00 and 07:30, as well as at three additional points during WE: 22:00, 00:00, and 01:30. The current analyses focused on PVT reciprocal reaction time (RRT: 1/RT) and the Stanford Sleepiness Scale (SSS).

Results: One–way ANOVA across WE timepoints (20:00, 22:00, 00:00, 01:30) revealed progressively slower reaction times as participants remained awake (20:00: 2.01±.32 [1/s]; 22:00: 1.84±.33; 00:00: 1.79±.29; 01:30: 1.71±.34; $F(3, 63)=13.78$, $p<.05$, $\eta^2= .40$). After recovery sleep, RRTs at 07:30 (1.69±.31) were slower than those at 07:30 after baseline sleep (1.95±.36) ($t(21)=5.46$, $p<.001$) and did not differ from 01:30 during WE ($t(21)=0.64$, $p=.53$), indicating a persistent impairment even after recovery sleep. SSS–indexed sleepiness progressively increased across WE (20:00: 1.77±.69; 22:00: 3.36±1.71; 00:00: 4.14±1.21; 01:30: 5.68±1.25; $F(3,63)=56.80$, $p<.001$, $\eta^2= .73$). However, unlike RRTs, morning sleepiness was significantly lower at 07:30 following recovery sleep, compared to 01:30 during WE ($t(21)=9.80$, $p<.001$).

Conclusion: Wake extension deteriorates vigilance and increases subjective sleepiness in young adolescents. However, like in adults, a 4h recovery sleep opportunity recalibrates perceived sleepiness while offering no benefit to objective vigilance. This incongruency underscores the vulnerability of youth to even partial sleep loss. Our next analyses will consider inattention phenotypes and recovery sleep physiology as potential moderators of these effects.

Clinical Implications:

Skin Motion Artifact of Radiopaque Bead Tracking Relative to Markerless Bone Tracking

2023 Lifespan Research Day Abstract Submission Contest

Research Category: Clinical & Translational, Basic Science
Primary Research Location: Suite 404, CORO West
Funded By: Dpt of Orthopaedics

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Abstract

Background: Skin–based markers are often associated with soft tissue artifacts (STAs) when used to study skeletal kinematics. The magnitude of error is a function of many factors (e.g., joint, type and dynamics of task, marker location). This study quantified STAs of the wrist during 7 motion tasks, as measured by skin surface marker displacement in comparison to markerless bone tracking using biplanar videoradiography (BVR), the gold standard for measuring skeletal kinematics.

Methods: Six subjects (female, 51–68 yrs) with no history of wrist or hand pathology were instructed to complete seven wrist motion tasks, where motion was measured through five radiopaque beads placed on the dorsal surface of the hand. An average of 400 frames were recorded per task. The 3D coordinates of the beads were tracked for all motion using XMALab. Wrist motion was defined as the motion of the third metacarpal (MC3) relative to the radius and was computed using BVR bone tracking software (Autoscoper). STA was defined as the displacement of the average of the beads relative to a neutral pose and reported as a function of wrist motion within each task.

Results: Bead displacement was greatest in the pitcher pouring motion task (~12.5 mm) and least in radial ulnar deviation (~4.5 mm) (Figure 1a). Grasping motion tasks had a larger average of bead displacement (~11.2 mm) than open hand tasks (~5 mm), likely due to the stretch of skin in prehension. Circumduction had the greatest number of outliers. At maximum ulnar deviation, subjects experienced maximum flexion. Average bead displacement was the greatest (~5 mm) at peak ulnar deviation and flexion (Figure 1b).

Conclusion: Grasping tasks yielded a higher bead displacement compared to open hand motion tasks. The large ranges of data for bead displacement and degree of rotation measurements indicate that the motion task was not uniformly performed by all subjects.

Clinical Implications: Quantification of STA will help to contextualize and perhaps enable computational accounting for artifacts, allowing for more–precise assessment of human skeletal kinematics by simply using noninvasive skin–based markers, rather than implanted radiopaque beads.

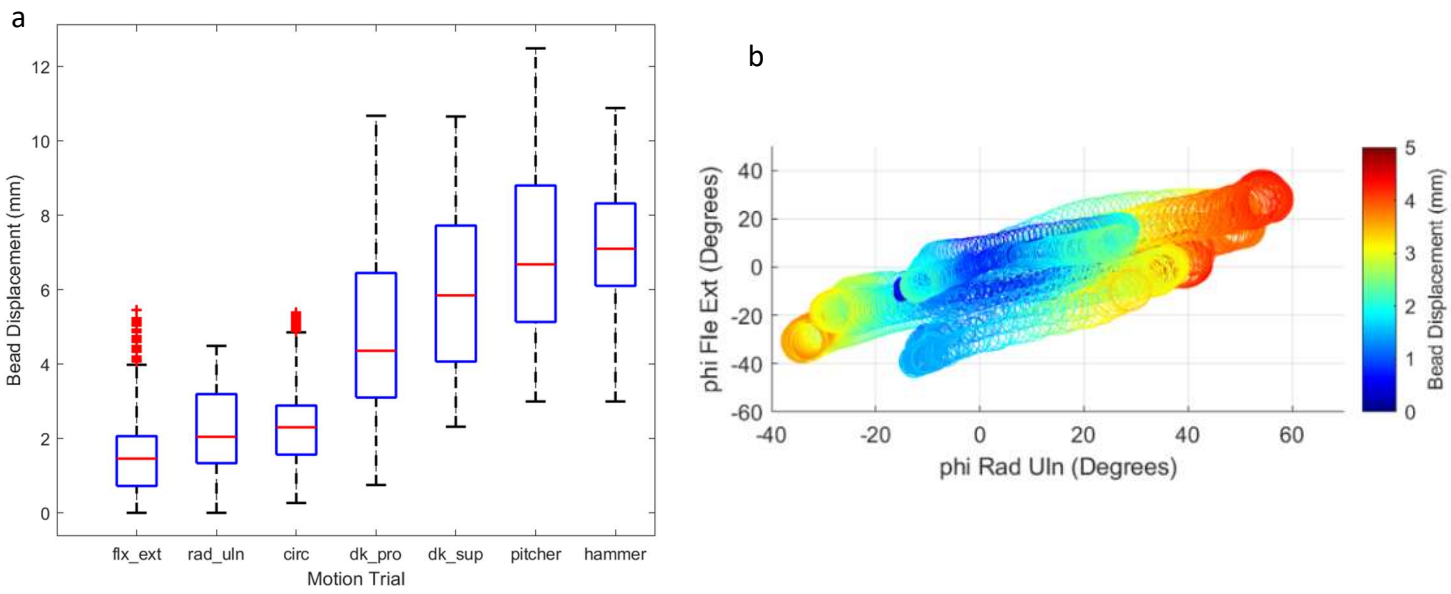

Figure 1. a) Box plot of the magnitude of average bead displacement for all subjects across seven motion tasks: flexion-extension, radial-ulnar deviation, circumduction, doorknob pronation and supination, pitcher pouring, and hammering.
b) Bead displacement at each degree of rotation (phi) for radial-ulnar deviation and flexion-extension wrist motion of all subjects (Radial-ulnar deviation motion trial)

Center of Biomedical Research Excellence (COBRE): Sleep and Circadian Methods Research Core

2023 Lifespan Research Day Abstract Submission Contest

Research Category: Clinical & Translational, Basic Science
Primary Research Location: Bradley Hospital
Funded By: P20 GM139743

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Abstract

Background: The goal of the COBRE Center on Sleep and circadian rhythms in child and adolescent mental health is to build a center that will help bridge the chasm between sleep and circadian science and child and adolescent mental health. The objective of the Sleep and Circadian Methods (SCM) Research Core is to support researchers in the appropriate use of sleep and circadian methods across the research process. Sleep and circadian data are complex and multimodal, requiring specialized expertise to select, acquire, score, analyze and interpret. The center helps by providing multidisciplinary resources focused on all aspects of the research process starting at study design moving through data acquisition and quality control to data processing and ending at analysis and interpretation. The Long-term goal of this core is to integrate pediatric sleep and circadian methods into Bradley Hospital infrastructure thus providing an enduring resource that will support research addressing the interplay between mental health, development, sleep, and circadian rhythms.

Methods: The aims of the SCM Research Core are 1) Support Center investigators in the selection, acquisition, scoring, analysis, and interpretation of sleep and circadian measures, 2) AIM 2: Facilitate access to in-lab facilities, instrumentation, software, and database resources, required for acquisition, storage, and scoring sleep and circadian data, and 3) Serve as resource for training in current best practices and for identifying novel methodological, measurement, and analytic approaches to sleep and circadian assessments suitable for pediatric mental health populations.

Results: We will report on the equipment that is available through the SCM Research Core, the number of consultations, trainings, and other support provided to Lifespan researchers, and the focus for the next year.

Conclusion: The SCM Research Core is an important resource to support research focused on sleep and mental health in pediatric populations.

Clinical Implications: Integrating sleep and mental health research will help improve mental health care for young people.

Comparing wrist acrigraphy to a novel wearable (Actigpatch): Nonparametric activity estimation

2023 Lifespan Research Day Abstract Submission Contest

Research Category: Basic Science, Innovation

Primary Research Location: COBRE Center for Sleep and Circadian Rhythms in Child and Adolescent Mental Health

Funded By: R01AA025593, P20GM139743. Actigpatches provided by Circadian Positioning Systems

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Abstract

Background: Wrist actigraphy is a standard for monitoring sleep in the field; however, data quality is reduced if participants remove the device. The Actigpatch is a novel, adhesive water−resistant wearable that we have previously demonstrated as comparable to wrist actigraphy for traditional sleep−wake estimation. Here we compare assessment of nonparametric activity indexes.

Methods: While following fixed schedules, 39 participants simultaneously wore the Micro Motionlogger actigraph (Ambulatory Monitoring Inc., Ardley, NY) on their non−dominant wrist and the Actigpatch (Circadian Positioning Systems, Newport, RI) over the triceps of their dominant arm. Our analyses included 35 participants (21F; 32.9±13.2yrs) who contributed =four nights of data (range: 4−14 [mean: 10] nights). After matching devices' tri−axial actimetry in one−minute epochs, we derived key non−parametric parameters of diurnal activity and calculated intraclass correlations to measure agreement. The non−parametric parameters include interdaily stability (IS), intradaily variability (IV), timing of the five hours of lowest activity (L5onset) and ten hours of highest activity (M10onset), and overall relative amplitude (RA).

Results: We observed agreement ranging from good for IS (ICC=0.77 [95%CI=0.59;0.88]; [all mean differences are patch−watch] mean difference=−0.21) to poor for IV (ICC=0.43 [0.12;0.67]; mean difference=0.29). ICC showed good agreement for M10onset (ICC=0.82 [0.67; 0.90]; mean difference=−37min) and excellent agreement for L5onset (ICC=0.91 [0.82;0.95]; mean difference=−2min). Finally, we identified good agreement when estimating the activity relative amplitude (RA ICC=0.86 [0.75; 0.93]; mean difference=0.03). An example of excellent agreement was manifested in the close estimation of L5onset (patch mean=1:04am, SD=70min; watch mean=1:06am, SD=68min).

Conclusion: Adding to our prior evidence that these two devices offer similar sleep−wake estimation using traditional algorithms, the present data indicate the Actigpatch offers good agreement to the Motionlogger for nonparametric analyses of IS, activity timing, and RA. Agreement was not as good for IV, with the Actigpatch showing more intradaily variability than the watch, perhaps due to the triceps placement. Because the Actigpatch is unobtrusive, water−resistant, and can be worn continuously for three weeks, it has potential benefits for studies of individuals who struggle with adherence to wearing wrist worn devices.

Clinical Implications:

Characterization of the Antimicrobial Mechanisms of Silver Carboxylate (AgCar) Released via a Titanium Dioxide/Polydimethyl Siloxane Matrix in the Context of Serratia Marcescens and Methicillin−sensitive Staphylococcus aureus

2023 Lifespan Research Day Abstract Submission Contest

Research Category: Clinical & Translational, Basic Science
Primary Research Location: Weiss Center for Orthopedic Trauma Research, Brown University, Providence, RI
Funded By: NIH/NIAID R25AI140490 (EIDS). Diane Weiss, the Sipprelle Family Foundation

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Abstract

Background: Antibiotic resistance continues to be an increasingly difficult and impactful challenge to modern healthcare. The development of novel antibiotics is time−consuming, and current antibiotics are vulnerable to antibiotic resistance due to poor stewardship and overreliance on synthetic antibiotics sharing a similar chemical structure. For this reason, silver, which has been shown to possess multi−mechanistic antimicrobial properties, is a potential alternative or synergist to current 'last resort' antibiotics. However, cytotoxicity concerns due to the uncontrolled release of silver have lead to an 'smart release' formulation of silver, silver carboxylate (AgCar) within a matrix of titanium dioxide and polydimethylsiloxane (TiO2/PDMS), which allows for controlled release of silver. While AgCar has been shown to be safe with predictable pharmacokinetics of release, the antimicrobial mechanism of action of AgCar has not been established. The aim of this project was to investigate the bactericidal mechanism of action of AgCar, including how it influences the release of reactive oxygen species (ROS) in Serratia marcescens and Methicillin−sensitive Staphylococcus aureus, two pathogens commonly encountered in orthopedic infections.

Methods: Bacteria were grown in 96 well plates and exposed to a gradient of AgCar ranging from 1x to 150x for 6 hours. 10nm and 30nm nanoparticle silver as well as 100% silver carboxylate with no TiO2:PDMS served as positive controls. 1% triton X and titanium dioxide/PDMS vehicle−only served as negative controls. To detect the general release of ROS, bacteria were lysed and read via colorimetry for levels of hydroxylamine oxidation using the abcam Cellular ROS Assay Kit.

Results: In Serratia marcescens, a 1x−30x concentration of AgCar produced a fold change of 1, or double the amount of ROS released when compared to the cell blank. AgCar 100x produced a 2 fold change and AgCar 150x produced a 3 fold change in ROS release, when compared to the cell blank. In Methicillin−sensitive Staphylococcus aureus, AgCar 1x−150x produced a fold change of 3, or a 300% increase in ROS production, when compared to the cell blank.

Conclusion: AgCar triggers the release of ROS in Serratia marcescens and Methicillin−sensitive Staphylococcus aureus in a more efficacious manner than nanoparticle silver, providing evidence for its bactericidal activity.

Clinical Implications:

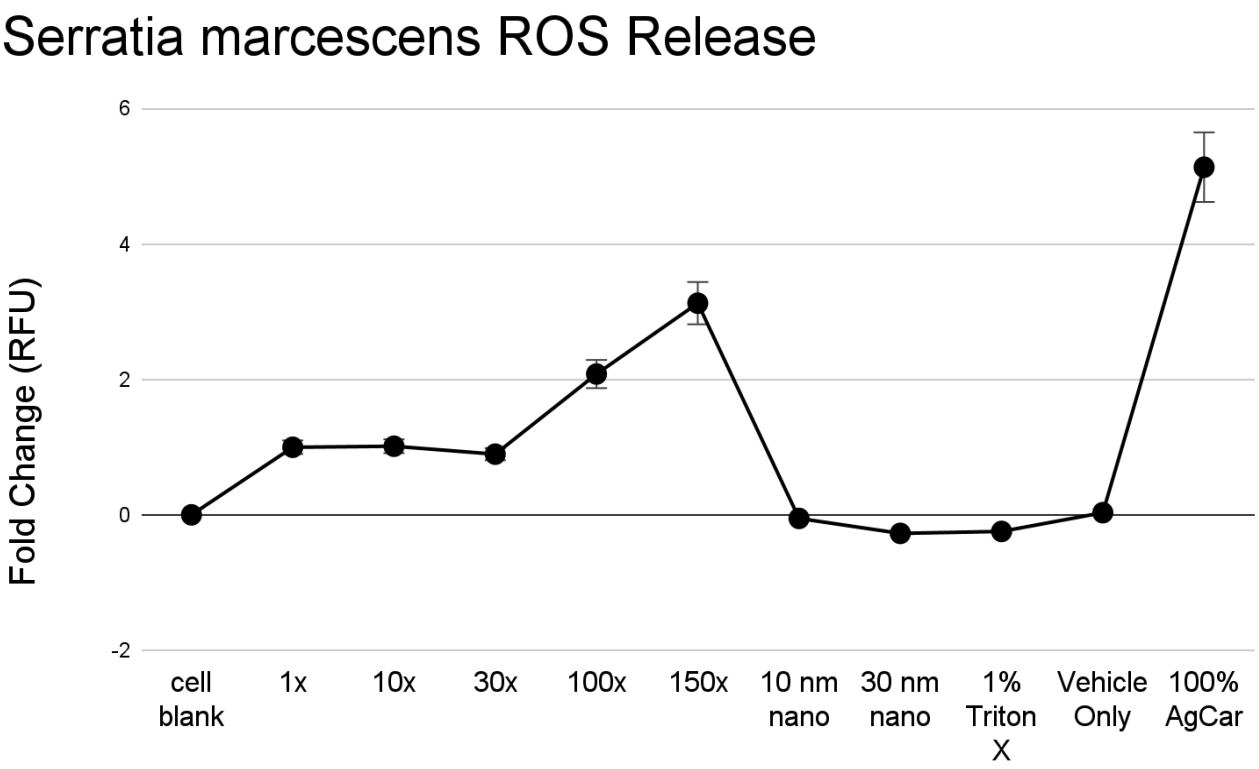

Figure 1. *Serratia marcescens* release of reactive oxygen species (ROS) in response to AgCar gradients.

Characterizing the Randomness of V(D)J Recombination in B and T cell Receptors

2023 Lifespan Research Day Abstract Submission Contest

Research Category: Basic Science

Primary Research Location: Rhode Island Hospital

Funded By: R35GM142638

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Abstract

Background: V(D)J recombination is the process through T cell receptors and B cell antibodies create diversity to match antigens. By analyzing the development of antibody receptor genetics as a multi−state system, the randomness of receptors can be characterized. When looking at disease states, there has been little research examining genetic changes in antibody production. We hypothesize that over the course of infection, there would is a measurable change in recombination of B cell antibodies and T cell receptors.

Methods: In this study, we analyze the randomness of genetic T and B cell receptors in peripheral blood. ImReP was used to characterize the sequence, number, and receptor chain. Shannon Entropy (sum of the probability of each element times the log of the inverse probably of each element) and Conditional Entropy (product of the probabilistic transition from each position in the sequence) were computed for each patient and chain.

Results: Approximately 2 million total and 80,000 unique reads were analyzed that matched B cell antibodies and T cell receptors. Analysis of conditional entropy found that the T cell receptor alpha sequences were the most entropic with the average log conditional entropy around 1 * 10^−19, Immunoglobulin Heavy chains were the second most entropic with an average log conditional entropy around 1 * 10^−18. Median conditional entropy for each chain was relatively stable across patients. Additionally, approximately the first 25% of each sequence is 100x more stable than the last 75% of each sequence.

Conclusion: Our results demonstrate that the majority of sequences in peripheral blood are stable across patients. However, there appears to be sequences, particularly in T cell receptor alpha and Immunoglobulin Heavy chains which have greater rates of randomness. The progression of randomness over the sequence might hint at whether additional randomness might occur.

Clinical Implications: Utilizing computational entropy may provide important information regarding patients' ability to produce antibody diversity over the course of infection. By determining changes in patients' sequence entropy, targeted treatment with antibody resources can be allocated correctly.

Staphylococcus aureus Biofilm Maturation Polarity

2023 Lifespan Research Day Abstract Submission Contest

Research Category: Basic Science

Primary Research Location: University of Rhode Island/ Kingston,RI

Funded By: Unfunded fellow research project

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Abstract

Background: Zeta (..) potential is a surrogate measure to estimate the surface potential of bacteria. Measuring charge repulsion effects we can determine the degree of electrostatic repulsions between antibiotics and biofilm. We aim to correlate the degree of polarity changes with corresponding biofilm maturation.

Methods: Using our previously described biofilm assay, six unique spa−type MRSA isolates were grown on tryptic soy agar. After overnight growth, a starting inoculum of 6−log10 CFU/mL was made in tryptic soy broth supplemented with 25mg/L calcium, 12.5mg/L magnesium, and 1.0% dextrose (STSB). ..−potential of planktonic cells (zero hour) was determined from the incolumn. Biofilm was then grown in tissue culture treated 12−well plates for 4 and 24 hours. At each time point, biofilms were sonicated and resuspended to measure ..−potential following biofilm production and compare it to its time zero counterpart (planktonic cells).Bacterial ..−potentials were measured using a Malvern Zetasizer Nano ZS (Malvern Instruments). ..−potentials were calculated from the electrophoretic mobility by Smoluchowski's equation at 25oC, with five repeats per sample. The dielectric constant of the dispersant set at 78.54. Viscosity set at 0.6864 cP and the refractive index at 1.333.

Results: All isolates displayed a negative ..−potential when in a planktonic state with mean charges of −4.3, −4.6, and −4.5mV for each high biofilm forming isolate and low biofilm forming isolates each respectively averaging −8, −6.8, and −4.7mV. All isolates demonstrated a significantly more negative charge ($p<0.05$) following biofilm formation than planktonic cells at all time points. While the greatest shift observed was from 0h to 4h, only high biofilm producing isolates continued this negative trend from 4h to 24h.

Conclusion: Biofilm production leads to an increase in net polarity from initial attachment through maturation.

Clinical Implications: Staphylococcus aureus is the most commonly identified organism in infective endocarditis (IE) and its ability to form biofilm on endothelial and prosthetic valves makes it a difficult pathogen to treat with antibiotics. Current guideline recommendations suggest polar antimicrobials, like daptomycin, for MRSA IE but note that further data is needed. The next step in improving anti−staphylococcal therapy is to measure these charge potentials, and the effects on formed staphylococcal biofilm.

Interpersonal Trauma, Shame, and Substance Use: A Systematic Review

2023 Lifespan Research Day Abstract Submission Contest

Research Category: Basic Science

Primary Research Location: Brown–Lifespan Center for Digital Health / Sexual Violence Prevention Lab at Brown U.

Funded By: N/A

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Abstract

Background: Interpersonal trauma is a simultaneous risk and transdiagnostic factor for a wide array of adverse mental health outcomes, including substance use; however, the influence of trauma is often multifaceted. Prior research has identified shame as a potential mediator in this complex, multidirectional cycle of substance use, interpersonal trauma, and related mental health outcomes, but further review is needed. As such, a systematic review was conducted to investigate this relationship and pinpoint its underlying processes within the existing literature.

Methods: Articles were collected using a Boolean search strategy of terms related to interpersonal trauma, substance use, and shame across six databases (n=4,063). Duplicates were subsequently removed (n=3,590). Researchers then conducted an abstract screening and full–text review for a final sample of 39 articles.

Results: Several articles had samples comprised only of women (n=17), and alcohol was identified most among the substances studied in the sample (n=27). Women and LGBTQ+ individuals were more likely to report a history of sexual assault, physical assault, and childhood sexual assault. The majority of articles identified shame as a mediator between interpersonal trauma and substance use; however, some articles found substance use to predate the onset of shame (notably, shame exclusively related to SUD, not interpersonal trauma)(n=9). Shame, alcohol use, and childhood abuse were predictive of abuse perpetration for men and women, with psychological abuse being the most common form of perpetration. Shame was also present following perpetration. Adverse mental health, isolation, and reluctance to disclose were either risk factors for or outcomes of shame and substance use. Resilience, self–regulation, and positive peer support were protective factors in preventing substance use and related maladaptive coping behaviors (e.g., self–harm, risky sexual behavior).

Conclusion: Future research should focus on highlighting underrepresented groups with interpersonal trauma exposure and compare outcomes of trauma–related shame and substance use–related shame to develop more informed interventions in this complex process.

Clinical Implications:

Cartilage−Derived Progenitor Cells Stimulate Meniscus Healing and Suppress NF−kB Pathway in Response to SDF−1

2023 Lifespan Research Day Abstract Submission Contest

Research Category: Clinical & Translational, Basic Science

Primary Research Location: Dept of Orthopaedics, Brown University/RI Hospital, Providence, RI, USA

Funded By: NIH NIAMS grant R21AR077326 and DOD grant W81XWH−20−1−0773.

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Abstract

Background: Meniscus injuries that fail to heal can instigate catabolic changes in the knee, posing a high risk for the development of post−traumatic osteoarthritis(PTOA). We have established human articular cartilage−derived progenitor cell−lines(CPCLs) as a potential therapeutic tool for accelerating meniscus tissue healing. Characterization of these cell lines revealed that they are less catabolic and hypertrophic than marrow−derived stromal cells(BM−MSCs). The Stromal Cell−Derived Factor−1(SDF−1)/CXCR4 pathway is crucially important for stimulating the directional migration of CPCs to stimulate meniscal fibrocartilage repair. In this study, our goal was to understand how SDF−1/CXCR4 signaling might play an influential role in helping CPCs achieve their therapeutic efficacy.

Methods: Healthy(non−arthritic) human CPC cell lines were established by enzymatic tissue digestion followed by stabilization using SV40 transduction. For in vivo studies, a radial tear was created in the outer third of the medial meniscus of skeletally mature athymic rats. In the cell treatment groups, 3.2X10^6 cells were injected into the joint capsule following meniscus injury. 7−weeks post−surgery, knees were isolated and histological studies were performed using Saf−O/Fast green stains. In vitro expression of cell signaling markers was determined by western blot analysis.

Results: Injection of CPCL significantly reduces the area of meniscal tear defect in rats as compared to injection of a BM−MSC line or untreated control groups(Fig.1). Further, we were surprised by our novel finding that SDF−1/CXCR4 pathway is regulated differently, between CPCs and a BM−MSC line. Our immunoblot results indicate that the CPCL, unlike BM−MSCs, demonstrate significantly more inhibition of canonical NF−..B(Fig.2A) and Erk(Fig.2B) pathways, as compared to untreated controls in the presence of SDF−1. The MAPK pathway is downregulated in both CPCL and BM−MSCs upon SDF−1 stimulation(Fig.2B).

Conclusion: Our results demonstrate that Intra−articular injection of CPC following meniscus tearing stimulates fibrocartilage restoration and healing. Further, CPCs and BM−MSCs respond differently to the chemokine SDF−1. Collectively, our results show a precise fine−tuning of an intricate downstream network of the SDF−1/CXCR4 axis that may govern how CPCs mediate chondroprotective anti−catabolic effects on the joint when they are injected as biologic therapy.

Clinical Implications: This study focuses on refining our understanding of molecular signaling that regulates cell−mediated meniscus healing and the prevention of PTOA.

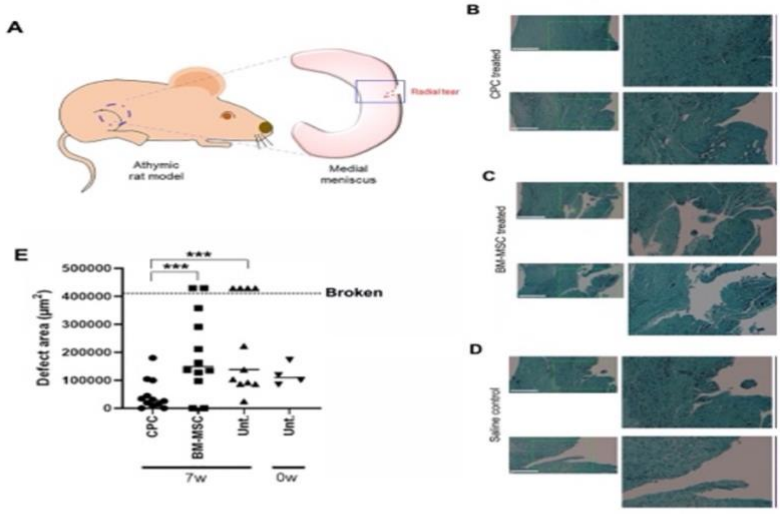

Fig – 1: Intra-articular injection of CPC line (CPCL3) following meniscus tearing stimulates fibrocartilage tear healing. ***(A)*** A radial tear was created in the outer third of the medial meniscus of skeletally mature athymic rats. Histology of meniscus tears 7 weeks following treatment with ***(B)*** CPCL3, ***(C)*** BM-MSCL, or ***(D)*** saline. ***(E)*** Open areas remaining within the meniscal tear channel were quantified by image analysis. Data points above the dotted line indicate menisci broken in two at the harvest time. Representative images in panels B, C, and D, were not obtained from these broken menisci samples. Scale bars represent 400 μm. N≥8 per group. ***, P≤0.005.

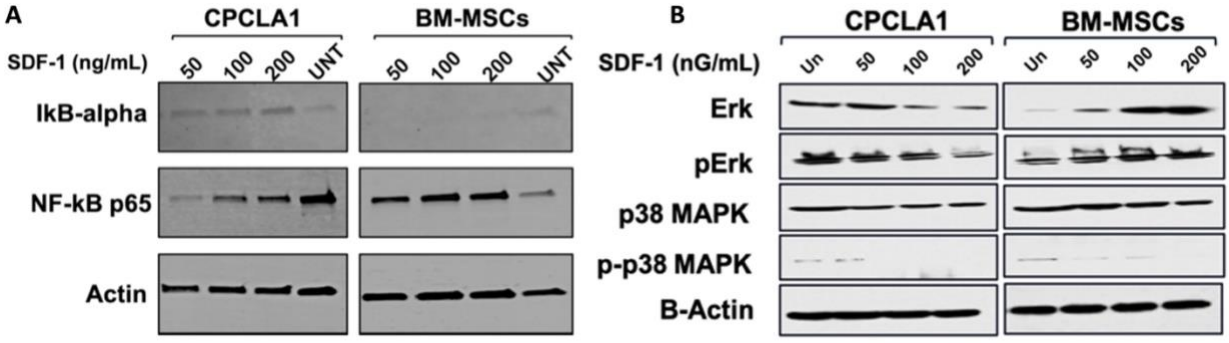

Fig – 2: ***(A)*** CPCs (left panel) and BM-MSCs (right panel) were treated with SDF-1. The activation of NF-kB pathway was determined by measuring the protein expression of downstream signaling molecules IkB-α and NF-kB p65, as well as their phosphorylation states. ***(B)*** CPCs (left panel) and BM-MSCs (Right panel) were treated with SDF-1. The activation of Erk and MAPK pathway was determined by measuring the expression of Erk and p38 along with their phosphorylation states (pErk) and (p-p38), respectively.

Accuracy and precision of markerless bone tracking relative to the gold−standard marker−based method

2023 Lifespan Research Day Abstract Submission Contest

Research Category: Basic Science

Primary Research Location: Bioengineering Research, Coro West

Funded By: NIH NIAMS K99/R00−AR069004; R01−AR047910; R01−AR074973 NIGMS P30−GM122732; Lucy Lippitt Endowment

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Abstract

Background: Biplane videoradiography is used to quantify 3D skeletal joint motion using either gold−standard marker−based or the more clinically applicable markerless tracking. Although highly accurate, marker−based tracking requires surgically implanting metal beads into the bone. Conversely, markerless tracking depends only on the bone shape but it is more sensitive to biplane system geometry and resulting bone−soft tissue image contrast. The aim of the current work was to quantify accuracy and precision of markerless tracking for a hop landing task.

Methods: A cadaveric knee specimen was used. Between 5−8 tantalum beads were implanted in the femur and tibia, and computed tomography (CT) scans were taken. The specimen was frozen and then moved through the calibrated biplane system field of view, simulating a hop landing motion. Marker−based tracking was completed by digitizing and tracking the displacement of the beads in the x−ray videos using specialized software (XMALab). X−ray videos and CTs were then processed to remove the spatial information associated with the beads. Markerless tracking was conducted by matching the markerless bone models to the x−ray videos using 2D−3D registration software (Autoscoper, Brown University). 3D knee motion was calculated from both marker−based and markerless tracking and expressed as 6 degrees of freedom (6DOF) kinematics − 3 rotations and 3 translations. For each DOF, mean (±SD) absolute difference between marker−based and markerless kinematics was used to quantify tracking accuracy. Bland−Altman tests were used to quantify bias and precision.

Results: Differences in flexion/extension, ab/adduction, and internal/external rotation were 0.10±0.06°, 0.50±0.14°, and 1.31±0.18° respectively. Translational differences in medial/lateral, anterior/posterior, and inferior/superior directions were 1.49±0.09 mm, 0.16±0.15 mm, and 0.10±0.03 mm respectively. Bland−Altman analyses revealed average biases of 0.61° and 0.58 mm with limits of agreement ranging from 0.22−0.34° and from 0.06−0.32 mm.

Conclusion: The accuracy, precision, and bias of 3D joint motion obtained from our hop landing biplane videography system configuration is dependent on the DOF of interest but are an order of magnitude superior to the precision of conventional motion capture approaches.

Clinical Implications: Internal/external rotation and anterior/posterior translation degrees of freedom evaluation with biplane videoradiography will be particularly relevant to evaluate anterior cruciate ligament injury and response to surgical treatment.

Characterizing the Antimicrobial Profile of Silver Carboxylate in Methicillin−Resistant S. aureus MW2/VRS1 − Derived Persister Cells

2023 Lifespan Research Day Abstract Submission Contest

Research Category: Basic Science, Innovation

Primary Research Location: Providence, RI

Funded By: P20GM103430/ P20GM121344, NIAIG R03AI159776, NIH/NIAID R25, NIH/NIAID R25AI140490

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Abstract

Background: Due to the misuse of antibiotics, surgical site infections (SSIs) by antimicrobial−resistant (AMR) pathogens are an increasing threat to the US healthcare system. Furthermore, the stagnant discovery of new antibiotics requires the development of novel approaches to combat these infections. Thus, research has turned to organometallics as a possible solution, specifically silver due to its multimodal bactericidal properties. To harness silver's capabilities, we have developed a silver carboxylate (AgCar) compound released via a titanium dioxide−PDMS matrix. In this study, we assess AgCar's ability to induce reactive oxygen species (ROS) release and peroxidase (POD) activity in Methicillin−Resistant S. aureus (MRSA) strains MW2 and VRS1 persister cells.

Methods: To generate persister cells, MRSA MW2 and VRS1 strains were grown to stationary phase overnight, then exposed to 20X and 4X antibiotic MIC respectively, to ensure the formation of persister cells. For ROS and POD assays, MW2/VRS1 persister cells were placed in 96−well plates and exposed to a gradient of AgCar from 1x to 150x for 6 hours. The levels of ROS and POD were assessed by the manufacturer's protocol for each of the respective kits. 10nm and 30nm nano−silver will serve as a positive control, and 1% triton X and titanium dioxide−PDMS matrix will serve as negative controls. Replicates of n=12 were done for each condition.

Results: When compared to untreated persister cells, MRSA MW2 persister cells treated with 30X−150X silver carboxylate demonstrated a statistically significant fold change increase in ROS release (100%−fold change, $P<0.05$), while VRS1 persister cells did not show statistically significant fold change in ROS release when treated with 1X−150X silver carboxylate. Peroxidase activity was not significantly different between treated and untreated persister cells.

Conclusion: Silver carboxylase demonstrates the potential for combating MRSA persister cells by inducing ROS stress without allowing bacteria to respond with appropriate antioxidant enzymes such as peroxidase. However, further research into the bactericidal mechanisms of silver carboxylase is required.

Clinical Implications: If properly harnessed, AgCar's multimodal antimicrobial capabilities have the potential to be an essential tool against AMR pathogens, especially when used synergistically with existing drugs.

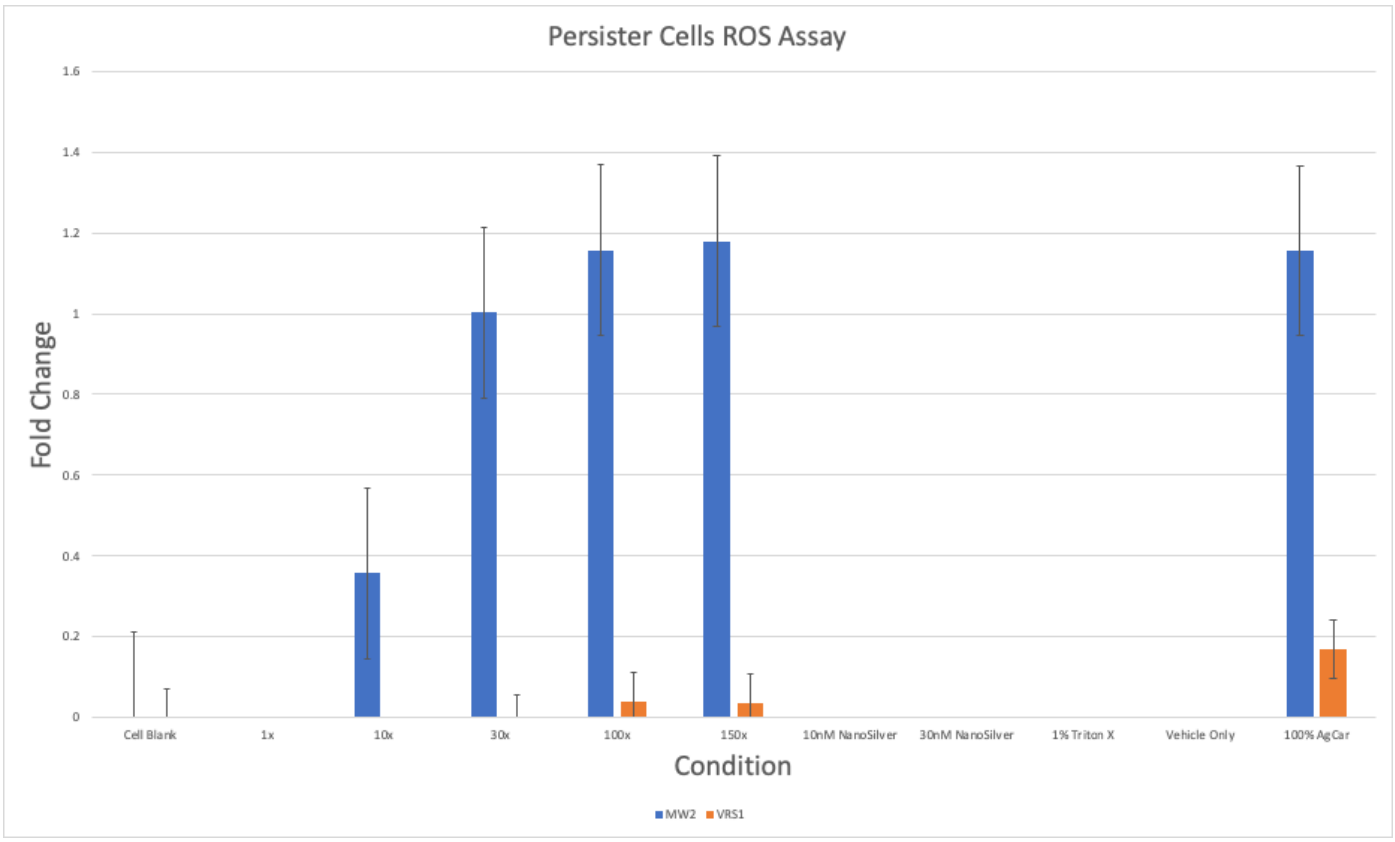

Figure 1. Fold change of ROS presence in persister cells of MRSA strains MW2 and VRS1.

Investigating Mechanisms of HOXB8−Conditional Neutrophil Progenitor Engraftment in the Murine Host

2023 Lifespan Research Day Abstract Submission Contest

Research Category: Clinical & Translational, Basic Science

Primary Research Location: Brown University and Rhode Island Hospital, Providence, RI USA

Funded By: R35GM124911

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Abstract

Background: Aim Neutropenia or neutrophil dysfunction are associated with increased susceptibility to severe bacterial and fungal infections. Recently, we characterized murine neutrophil progenitor cell lines (NPs) that are conditionally immortalized via HoxB8 expression and are uniquely capable of engrafting in the naïve murine host. We propose that NPs may serve as a therapeutic adjunct for reducing infection resulting from neutropenia or neutrophil dysfunction. To achieve this, it is first important to understand the mechanisms of NP engraftment in the hematopoietic niche. We have observed that NPs home and/or engraft via a VLA4−independent, beta1 integrin−dependent mechanism. We found that engrafted NPs proliferate and differentiate into mature neutrophils that are mobilized to the periphery via canonical CXCR2 signaling. Here, we describe studies to determine the impact of cytoreductive conditioning of host niche space via antibody−mediated depletion of Ly6G−expressing cells or busulfan−mediated HSPC ablation on NP engraftment. To further evaluate the potential translational utility of NPs, we also probe candidate integrin alpha subunits and signaling receptors to determine their role in NP homing and engraftment.

Methods:

Results: Mice depleted of neutrophils via anti−ly6G treatment prior to NP transplant did not result in increased engraftment capacity as measured by frequency or cell number compared to control mice. NP mutants which lack expression of integrin alpha 5 had reduced frequency and cell count in murine host bone marrow post competitive adoptive transfer compared to parental NPs.

Conclusion: Conditioning host bone marrow via anti−ly6G depletion of host neutrophils does not impact engraftment capacity of NPs suggesting that increased niche space created via cytoreduction specific to neutrophils do not increase engraftment capacity of NPs. Results demonstrate that integrin alpha 5 plays a role in NP engraftment capacity in the bone marrow and potentially plays a role in proliferation capacity once cells lodge in the bone marrow niche.

Clinical Implications: Engineered neutrophils, in theory, could be harnessed to treat cases of sepsis caused by traumatic injury to the lungs or peritoneal cavity and in cases of acute neutropenia, which results in high−risk vulnerability of infection induced by myeloablative conditioning for hematopoietic stem cell transfer (HSCT) efficacy.

Responsiveness of ACL Cross Sectional Area to Oral Relaxin Administration

2023 Lifespan Research Day Abstract Submission Contest

Research Category: Basic Science

Primary Research Location: RIH/Brown University Bioengineering Laboratory

Funded By: NIH NIAMS K99/R00–AR069004; NIGMS P30–GM122732

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Abstract

Background: Anterior cruciate ligament (ACL) injuries are more common in females than males with hormonal fluctuations speculated to contribute in some way. The hormone relaxin has been demonstrated to have negative effects on collagen in vitro and has a higher circulating concentration in females. For these reasons, the overarching project goal is to determine whether oral relaxin administration induces ACL biomechanical laxity in a rodent model. To achieve this, the aims of the present study were to develop an optical method to quantify ligament cross–sectional area (CSA) that enables calculation of ACL mechanical properties, and to explore whether CSA was affected by age and/or relaxin treatment.

Methods: Four Sprague–Dawley rats were used to develop the optical method for CSA measurement. All soft tissue was dissected leaving the femur–ACL–tibia complex which was mounted in a custom loading jig. Sagittal and coronal images of the ACL were taken, and the ligament diameters were measured digitally at their smallest width using ImageJ. CSA was calculated by fitting an ellipse to the measures. Intra– and inter–rater agreement was expressed as the mean absolute and percent differences. Bias and precision were tested using Bland–Altman analyses. For the overarching study, 36 female 10–12 week old Sprague–Dawley rats were randomized into 3 groups of 12: 1) no treatment; 2) treated with relaxin for 10 days; 3) aged to 14–16 weeks old. CSA results were described qualitatively.

Results: Intra and inter–user agreement in CSA ranged from 0.3–1.1mm2, equivalent to 1–9.9% of the largest CSA measured. Intra– and inter–user bias ± precision was 0.001±0.04mm^2 and −0.04mm2±0.05mm^2, respectively. Mean CSA (±SD) for the three experimental groups was 0.75±0.18mm^2, 0.76±0.14mm^2 and 0.76±0.16mm^2, respectively.

Conclusion: Based on existing reports of rat ACL biomechanical properties, the magnitude of differences in intra– and inter–user optically–measured CSA agreement will not overshadow expected experimental differences. The similarity in CSA across the 3 experimental groups suggests that any differences in biomechanical properties of the ACL will be attributable to changes in the underlying material property of the tissue and not CSA morphology.

Clinical Implications: If relaxin administration diminishes ACL biomechanical properties, future treatments that target relaxin could reduce female ACL injury risk.

Role of CHSY3 in Cartilage Homeostasis and Carpometacarpal Joint Osteoarthritis

2023 Lifespan Research Day Abstract Submission Contest

Research Category: Basic Science
Primary Research Location: The Orthopaedic Research Laboratories, Coro Center West, 1 Hoppin St, Providence, RI
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Abstract

Background: Carpometacarpal joint osteoarthritis (CMCJ OA) is a joint degenerative disease of the hand and the etiology of CMCJ OA remains elusive. A study by Jurynec et al. identified cohorts of patients with CMCJ OA that segregated as an apparent autosomal dominant trait. This study has linked CMCJ OA to a rare coding variant for the gene encoding chondroitin sulfate synthase 3 (CHSY3). This variant is a point mutation causing a substitution of glycine to arginine (G629R) in a region that participates in the production of chondroitin sulfate, suggesting that an alteration in this process is a major risk factor for the development of OA. The goal of this project is to develop novel cell lines that either lack CHSY3 or express the CHSY3 G629R variant, and then study the impacts of the CHSY3G629R mutation on chondrocyte biology.

Methods: Acan+ murine chondrocytes were derived from the knee joint of AcanCreER;Ai9 mice and immortalized with the retroviral expression of SV40 large T antigen. To knock down CHSY3 expression, GFP−bearing gRNA−CRISPR−Cas9 constructs were used to transiently transfect the cells. Positive transfectants were sorted by FACS and single cell clones were obtained by limited dilution and screened by western blotting for CHSY3 ablation. DDK−tagged murine wildtype CHSY3 cDNA was obtained by cloning, and the CHSY3 G629R mutation was introduced using standard PCR−based mutagenesis approaches. Wildtype CHSY3 and CHSY3 G629R cDNA were cloned into pcDNA3 and pLenti expression systems for transient and stable expression analysis. The impact of CHSY3 G629R mutation on its enzymatic activity, the anabolic and catabolic gene expression, and chondrocyte proliferation and survival will be determined via biological and biochemical analyses.

Results: Constructs for stable transfection containing the mutant CHSY3 gene were produced and confirmed by sequencing to contain the point mutation. Several clones are now being screened for successful CHSY3 knockout by Cas9.

Conclusion: Currently, the cell lines being screened already show morphological differences from the wild−type. Once a knockout line is isolated, the CHSY3 protein can be better understood.

Clinical Implications: May shed light on the mechanistic cause of this familial version of thumb CMCJ OA, as well as other forms of the disease.