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Perpetua Staff

Letter from the Editor

It is with great pleasure that I present the latest issue of *Perpetua* to you, the students, faculty, researchers, and associates of the University of Alabama in Huntsville as well as the curious minds outside the UAH system. On behalf of *Perpetua*, I would like to extend a sincere expression of gratitude for the generous contribution of all the graduate students, faculty, administrators, and alumni who have given their time towards the growth and realization of this publication.

Perpetua has now, for three years, been driven with a mission to represent the excellency of the undergraduate research community. A great but worthy responsibility. We are indebted to the researchers who have entrusted their work to us and extended their confidence to represent their research in the best possible manner.

Since the Fall of 2016, *Perpetua* has provided a platform for researchers to release their work to the public. This journal is populated by the students and faculty of this university who understand that this journal serves a greater purpose than broadcasting the accomplishments of an undergraduate community. These publications help kindle a spark of interest for so many readers. To be dreamers. Scientists. Pioneers. To be explorers of a wilderness of innumerable, new, and nameless discoveries. To be curious. Or wild in the noblest sense.

However, the hard work of these researchers is not to be trivialized with emotional platitudes. Research demands patience, planning, and determination. As you sift through these pages, we hope that you yourselves are inspired to pursue your own research and join this talented community of undergraduates at UAH.

Per Angusta Ad Augusta,

Benjamin Tran
Editor-in-Chief
Perpetua

Special Thanks

Perpetua is a collaborative effort and publication would be impossible without the support of numerous individuals and organizations across UAH and throughout the greater Huntsville research and outreach community. We offer special thanks to all who have contributed their time, expertise, financial support, and hard work to *Perpetua*. A few of our biggest contributors are recognized below.

First and foremost, we would like to thank the undergraduate student researchers for entrusting us with the privilege and the responsibility of promoting their work. We thank the various faculty and staff who serve as sponsors to undergraduate research and to the Research and Creative Experience for Undergraduates for providing resources and opportunities and who likewise support and promote undergraduate research.

We thank the Office of Student Life for providing ample opportunities to promote Perpetua and its purpose. We thank the Office of Academic Affairs for enabling us to reach as many members of the UAH community as possible. We would also like to thank Dr. William Wilkerson and the UAH Honors College for their commitment to providing additional financial support to *Perpetua*. Next, we would like to extend our thanks to our faculty advisors: Mr. David Cook, Dr. Yu Lei, and Dr. Hamsa Mahafza, who have consistently provided exceptional insight and guidance to our editorial staff since our inception.

Finally, we thank every UAH graduate student and faculty member who served as a reviewer for one of the manuscripts featured in this issue. Without such individuals volunteering their time and expertise, *Perpetua* would not be able to provide our services to the UAH community.

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Sport Performance Measures in Youth Wheelchair Basketball Athletes

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Abstract – Wheelchair basketball is an adapted sport in which individuals of all ages participate. Sport specific measures are important for training and to compare sport performances between players of various levels. Baseline values have been established for elite players, but they have not been established for amateur athletes. **PURPOSE:** To determine baseline measures in sport performance for youth wheelchair basketball athletes. **METHODS**: Four males and one female (age = 15 ± 1 year) were recruited from a local high school wheelchair basketball team. Body composition (3-site skinfold) and anthropometric measures (body mass, wingspan, and shoulder width) were evaluated, along with performance testing measures (pick-up test, max pass,10m sprint with and without basketball, spot shot test, and t-test). **RESULTS**: Athletes all had spina bifida, and either Junior 1 or Junior 2 classification per the National Wheelchair Basketball Association. Mean scores were reported for each measure taken: body mass (73.6 ± 44.94 kg), body composition (31.6 ± 22.28 percent body fat), wingspan (168 ± 16.88 cm), shoulder width (34.4 ± 3.78 cm), pick-up test (20.32 ± 2.80 cm), max pass (2.86 m ± 1.18 m), sprint with ball (4.09 sec. ± 0.45 sec), sprint without ball (3.40 ± 0.40 sec), spot shot (29.6 ± 11.10 points), and t-test (20.48 ± 3.05 sec). **CONCLUSIONS**: These scores provide feedback to players on their level of wheelchair basketball performance compared to other players on the same, or various, levels along with showing areas that need to be focused for improvement during practice.

Keywords: wheelchair basketball, performance measures, amateur, youth

I. Introduction

Wheelchair basketball is an adapted sport that continues to increase in popularity across the world (International Wheelchair Basketball Federation, 2018). The sport began after wounded World War II veterans needed an outlet for exercise and wheelchair basketball gave an alternative to the standard version of basketball that they could no longer perform (National Association of Wheelchair Basketball, 2018). The sport has since evolved to be a world-renown sport in which over one-hundred countries are participating. Over the past decade, it has received more publicity, and more people than ever are participating in the sport. Wheelchair basketball uses similar rules to able-bodied basketball, but adaptations are made to assist individuals using a wheelchair and to accommodate different levels of disability. The players are ranked based on the severity of their disability on a point scale that goes from 1.0 (minimal functional potential) to 4.5 points (maximal functional potential) (Cavedon, Zancanaro, & Milanese, 2015; IWBF, 2018). Since each player has a different classification level, they will have different athletic abilities and potential results during game play.

The sport of wheelchair basketball requires the players to perform repeated short, intense exercise bouts including rapid sprints, acceleration, and decelerations (Coutts, 1992; Yanci et al., 2015). Based upon the demands of the game, players must utilize muscular strength, muscular endurance, and cardiovascular endurance during game play. Sport specific testing measures can indicate the areas of sports performance an athlete excels in and the areas needing additional practice (Groot, Balvers, Kouwenhoven, & Janssen, 2012). Professional wheelchair basketball athletes have mastered the sport specific skills with years of practice and playing competitive games (Groot et al, 2012). One study was performed to determine the performance measures in professional athletes to establish baseline measurements for success (Vanlandewijck, Spaepen, & Lysens, 1995). By reviewing this study, it gives a baseline of which performance tests are used in higher level athletes to compare to younger athletes. However, youth athletes are just beginning to zone in on the skills needed to be successful in the sport.

Although the demands placed on youth players are similar to professional athletes, baseline sports performance levels have not been established for youth wheelchair basketball players.

There are different types of sport testing that coaches and athletes can use to determine physical fitness and sport related skills such as the t-test, sprints, and cardiovascular testing. Each test is based on different aspects of the sport including speed, agility, and cardiovascular endurance. Several studies have been performed to determine validity and reliability of these types of tests for elite wheelchair basketball athletes (Cavedon et al., 2015; Groot et al., 2012; Yanci et al., 2015). Baseline sports performance measures are used to make sure each athlete is in good health and can withstand the demands placed on him or her with minimal risk of injury. Amateur youth athletes are not exposed to these tests until later in their career due to lack of testing measures or accessibility to these tests at their current level. By using specific field tests that measure the imposed demands from the sport, the performance measures of each player can be evaluated and show how they compare to higher level athletes. It is useful to be able to compare values between youth and elite athletes to allow the youth athletes to determine if they are physically and skillfully able to compete in higher levels of wheelchair basketball competition. The information obtained can also be used to modify conditioning sessions and practices to improve skills that may be lacking from individual players. The purpose of this study was to determine baseline sport performance measures in amateur wheelchair basketball athletes.

II. Methodology

Participants

Prior to participation in the study, informed assent was obtained from each participant and their legal guardian. A total of five amateur youth wheelchair basketball athletes were recruited for the study. The participants were between 14 and 16 years of age, with four being male and one being female. Participation in the study consisted of those athletes that had less than five years of participation in wheelchair basketball. All players were either Junior1 (J1) or Junior 2 (J2) classified. The National Wheelchair Basketball Association (NWBA) classification is based on function of each athlete (2018). The J1 classification of a player includes the International Wheelchair Basketball Federation (IWBF) classes of 1.0, 1.5, 2.0 and 2.5. These players have limited vertical and forward stability of the trunk and commonly rely on passive stabilization of the trunk. The J2 classification for players consists of the IWBF classifications of 3.0, 3.5, 4.0, and 4.5. These players have active control of their trunk in both the forward and vertical planes (NWBA, 2018). The IWBF classifications are different from junior classifications: "the players are assigned classifications ranging from 1 to 4, with 0.5 for exceptional cases which do not fit exactly into one class, and the 4.5 category for the player with least or minimal disability" (IWBF, 2018). Classification for these players are observed in their competition wheelchairs, in a training situation before the tournament commences, and then in an actual competition game, at which their classification will be confirmed or modified if the classification panel feels it is necessary.

Accommodation Session

Prior to any sports performance testing, each participant went through a two-hour accommodation session. During this time, all tests were demonstrated, and all participants performed a walk-through trial of each skill. This gave the participants an opportunity to become familiar with the tests and a chance to ask any questions prior to the study commencing.

Skinfold measurements: The first measurements taken were the skinfold measurements from each participant. A total of three skinfold measurements using Lange skinfold calipers (Santa Cruz, CA) were taken at the subscapular, triceps, and chest locations for the males and the abdomen, subscapular, and triceps for the female athlete. The measurements were taken a total of two times at each site, and if the measurements were not within two millimeters of each other, a third measurement was then taken. The average of all sites was then recorded based upon standard ACSM protocol (Riebe, Ehrman, Liguori, & Magel, 2018). Once the skinfold averages were determined, all values for the participants was analyzed by the three-site Jackson and Pollock formula to determine body density. The body density values were then used to compute overall body fat for each participant $\left[\left(\frac{4.95}{Db}\right) - 4.50\right] * 100$ (Riebe et al., 2018).

Wingspan and shoulder width: Wingspan and shoulder width were measured with a Gulick tape measure (Gays Mills. WI). The wingspan measure (Gays Mills. WI). The wingspan measurement was taken by asking the participants to spread their arms out straight and using a Gulick tape measure to measure from the tip of the middle finger on the right hand, going across the shoulder blades and to the tip of the middle finger on the left hand ("What's Your Wingspan?", 2019). The shoulder width measurement was determined by measuring the distance from the acromion process of the participant's right shoulder to the acromion process of the left shoulder (performed on the anterior aspect of the athlete). The wingspan and shoulder width measurements were recorded in centimeters.

Pick-up test: The pick-up test was used to determine flexibility and speed. From a stationary position, on one side of the court, the participant propelled forward and picked up four basketball balls (Winnipeg, MB, Canada) from the floor, two times with the left hand and two times with the right hand. The balls were placed at random locations on the court. After picking up the ball, it was placed in the lap of the participant, and the wheelchair was pushed once before throwing the ball. Once all the balls were picked up, the participant returned to the start line. The total time taken to complete the test was recorded using the Brower TCi Timing Gate (USA), with the time starting when the participant's front wheels cross the gate and ending when the front wheels cross the gate a second time. All participants performed the test three times with at least three minutes of rest between trials. The fastest completion time of the three trials was used for results. The fastest time was used to follow pick-up test standard protocol.

Max-pass test with a 5kg medicine ball: The max-pass test was used to measure upper body strength. Participants were placed behind a marked line on the floor with their chair pushed up against a wall to prevent rolling. The participants were instructed to chest pass the medicine ball for maximum distance. The spot where the medicine ball contacted the floor was marked. The distance between the mark and the pass line was measured using a measuring tape. Each participant performed a total of 3 trials of the test with at least 2 minutes of rest between trials. The distances were recorded, and the average of the 3 trails was used for the results. Averages were used across athletes to follow standardized protocol for the max-pass test.

10m sprint with and without a basketball: This test was used to determine speed and dribbling ability. All sprint times were measured using the Brower TCI Timing Gate System (USA). The timer started when the participant broke the laser beam that is passed between the two gates. Once started, the participants sprinted at maximal speed until they crossed the second gate located 10 meters away. Each participant performed 3 trials with a basketball and 3 trials without a basketball. During the trial with the basketball, participants were required to bounce the ball once per 2 pushes, as is the rule set by IWBF (IWBF, 2018). If the participant did not adhere to these rules, they had to restart the trial. Participants were given a 2-minute rest period between each sprint set and a 5-minute rest between the two trials. The fastest time out of the three trials for each test were used for data analysis. The fastest time was kept ensuring accurate protocol for sprint testing.

Spot-shot test: The spot-shot test was used to determine shooting skill and agility on the court. For the spot shot test, the players performed a total of five shots from four positions around the lane (i.e. the area between the free-throw line and the baseline), two at the top of the lane (left and right), and two at the base of the lane (left and right) for a total of twenty shots. This test was for shooting accuracy only, so the participants were not timed during the test. Depending on where the ball hit the scoring board, players accrued points. A total of 3 points were scored when the basket was made, 1 point when the ball touched the cylinder but did not go in, and 0 points were scored when the ball did not touch the cylinder at all. The score was the sum of the points from the 20 shots (final scores ranging from 0 to 60 points). This protocol was established from previous studies using the spot shot test (Groot et al, 2012).

T-test: The t-test was used to determine speed and agility (**Figure 1**). The participants were instructed to begin at cone A and then sprint to cone B, touching it with their right hand. Next, they sprinted over to cone C and back across to cone D. The players then sprinted back to B and finished at cone A. The participants were only allowed to make forward movements during the test. Each participant completed three separate trials with a 2-minute rest period between the trials. All times to completion were recorded using the Brower TCi Timing Gate (USA), and the average of the three times was used for data analysis.

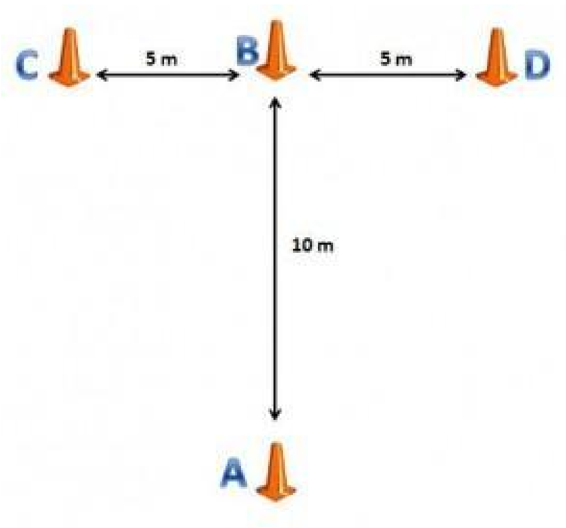

Figure 1. *T-test for Agility*

III. Results

Table 1. *Individual Participant Descriptive Statistics*

Participant	Age (yrs.)	Shoulder Width (cm)	Wing-span (cm)	Weight (kg)	NWBA Classification	Body Fat (%)
1	15	36	175	44.2	J1	15.0
2	15	29	139	54.4	J1	31.0
3	16	37	181	62.0	J2	32.0
4	14	32	177	54.2	J1	12.0
5	15	38	168	153.2	J1	68.0

Note. All participants had diagnosed Spina Bifida; S.B. = Spina bifida; J1 = Junior 1; J2 = Junior 2; NWBA = National Wheelchair Basketball Association.

Table 2. *Individual Wheelchair Basketball Performance Measures*

Participant	Max Pass (meters)	Sprint w/ Ball (sec)	Sprint w/o Ball (sec)	T-Test (sec)	Spot Shot Points	Pickup Ball (sec)
1	2.69	3.82	3.36	17.84	42	17.60
2	1.08	4.59	3.77	22.81	18	23.79
3	3.57	3.58	3.14	19.15	41	18.20
4	2.74	3.92	2.90	18.01	23	19.20
5	4.24	4.52	3.81	24.60	24	22.80

A total of five participants fully completed the study and the athletes ranged between 14 and 16 years of age with all participants having physician-diagnosed spina bifida. Athletes were either J1 or J2 classified. **Table 1** shows all ages, shoulder width, wingspan, weight, disability, NWBA classification, and body fat percentages for all of the participants. The mean shoulder width measurement was 34.4 centimeters (SD ± 3.78) and the mean wingspan was 168 centimeters (SD ± 16.88). The average weight for the participants was 73.6 kilograms (SD ± 44.94) and the average amount of body fat was 31.6 percent (SD ± 22.28).

Individual performance variable scores are listed in **Table 2**. The max pass mean was 2.86 seconds (SD ± 1.18). The average time to completion of the sprint with a basketball was 4.09 seconds (SD ± 0.45), while the sprint without a basketball exhibited a mean of 3.40 seconds (SD ± 0.40). The t-test average time to completion was 20.48 seconds (SD ± 3.05); Spot shot test average score was 29.6 points (SD ± 11.10) and the pickup ball average time to completion was 20.32 seconds (SD ± 2.80).

IV. Discussion

The current study showcased a variety of sport performance tests completed by youth wheelchair basketball players, to determine overall skill level. Body composition and anthropometric data from each player was collected including age, shoulder width, wingspan, body mass, type of disability, NWBA classification, and percent body fat. The specific tests were chosen to determine the five main sport performance areas necessary for success in wheelchair basketball: agility, flexibility, strength/power, and sport specific skills.

Body Composition & Anthropometric Measures

Body fat percentage and body mass allowed us to determine the health-related fitness characteristics of each participant. Participation in wheelchair basketball can affect body composition as well as the level of sport classification due to the amount of active time performed while a participant is in their chair. Functionality determines what participants can and cannot do. Athletes who have higher mobility tend to have less amounts of body fat than athletes who are just starting out or who are less functional (Cavedon et al, 2015).

These measures along with shoulder width, wingspan, and classification can affect performance in wheelchair basketball (Yanci et al, 2015). Having an increased shoulder width and wingspan can positively impact flexibility testing, as needed in the pick-up ball test, since a longer reach allows for less flexion at the trunk to pick up the ball from the court (Cavedon et al, 2015). Longer wingspans could lead to better performance score on testing for any player but especially for players who have limited flexion at the trunk. Flexibility is a vital part of the classification of a youth player and is used to determine whether a player is a J1 or J2. Since the athletes are still growing, wingspan and shoulder width can and most likely will change over time. It is important to measure this periodically due to it impacting test scores. Although these measurements can't be practiced, they are important measurements that can be helpful in sports performance and potential advantages in the sport of wheelchair basketball.

Sport Performance Measures

The pick-up ball test was used to measure a participant's flexibility agility (Yanci et al., 2015). A player must be flexible enough to bend at the trunk and pick up the ball from the court, along with being able to rebound the ball from the backboard. To pick up the ball, a player can either reach over and directly pick it up off the floor or use a wheel to maneuver the ball up closer to their trunk. This is done by wedging the ball between the hand and wheel and using the wheel's rotary motion to bring the ball upward (BC Wheelchair Basketball Society, 2019). As in the Cavedon et al. 2015 study, the players with longer wingspans or shoulder width measurements had a faster time for the pick-up ball test, due to the ease of reaching the ball to pick it up off the floor. Using this test for youth athletes helps to determine the basic skill level of the players involved in wheelchair basketball. If a player cannot pick up the ball from the court, they are at a serious disadvantage and would affect their ability to be competitive in the sport.

Muscular strength and power are important components of wheelchair basketball performance because a player needs to be able to produce enough power to propel themselves down the court and pass the basketball far distances. To achieve this, each player needs to have enough upper body strength to move his or her body weight just as quickly as other players (Yanci et al., 2015). The medicine ball max pass test was used to measure upper body strength for each participant. The max pass allowed us to use a sport specific skill to measure the upper body strength because the athletes utilize a basketball chest pass for the test (Gil et al., 2015). Body composition percentage may not always accurately predict muscular strength abilities but can be linked to classification and functionality (Gil et al, 2015). This can be seen by comparing body composition values to max pass scores in **Table 1** and **Table 2**. An individual with a higher percent of body fat and body mass must utilize more muscular strength to travel a set distance compared to a player with a lower body mass and less overall body fat (Gil et al, 2015; Tonson et al, 2010). Youth players typically have lower levels of upper body strength. By using this data, coaches and players can see if their strength is progressing throughout the season or if incremental increases are seen following participation in a strength and conditioning program.

The 10m sprint with and without the basketball also includes a power and muscular strength component. The participants had to exert maximal force upon the wheels to propel themselves forward until they crossed the 10m line (3.40 ± 0.40 sec). The 10m sprint with the basketball measures power output during the sport specific skill of dribbling the ball. Completion for the 10m sprint with a ball (4.09 ± 0.445 seconds) resulted in time values that were slower than sprints performed without the ball (3.40 ± 0.40 seconds). The researchers believe that the slower sprint times were due to the players not being able to use max power output on each push from having to dribble and balance the basketball simultaneously. In another study, it was found that the time difference was indicative of years of experience in playing basketball and everyday wheelchair use (Gil et al, 2015). If a player is more adapted to using their chair, he or she can maneuver more efficiently; furthermore, if he or she has played for a few years, there is higher adaptation to dribbling the ball. Being able to sprint quickly with or without the basketball gives an advantage to those players. If they are able to maneuver down the court quicker than the opponents, it opens up opportunities to shoot the ball without as many defensive players around the player. It also works for the player when they are on defense, the faster they can make it down the court, the better defensive position the player can be in.

Muscular strength, flexibility, and agility are important components of wheelchair basketball, and can help players be able to score points by shooting the basketball. The spot-shot test allowed us to test the shooting accuracy of the youth wheelchair basketball players from different locations around the court. The data shows a strong positive relationship between the strength output from the max pass test and spot-shot performance scores, which was also seen in the study performed by Cavedon et al. (2015). To be well rounded players, athletes need to be able to shoot and make a basket from various locations on the court. When players can only make a shot closer to the goal, it makes it increasingly difficult to get there when dealing with defensive players on an opposing team. Data collecting during this test provides evidence into how well a player can accurately shoot, and also determine if they need to work on upper body strength.

The t-test measured the participant's agility by incorporating forward movement sprints with pinpoint turning. A player must be able to maneuver quickly as if the chair is an extension of their body. The mean score for the t-test was 20.48 ± 3.05 seconds. In a previous study conducted by Gil et al. (2015), elite male wheelchair basketball players averaged a completion time of 16.51 ± 1.02 seconds for the t-test. The results of the study indicate that the amateur players have slower times to completion by around four seconds. This could be due to less playing time and practice to improve agility (Gil et al, 2015; Vanlandewijck, Verellen, Beckman, Connick, & Tweedy, 2011). The t-test can be used regularly throughout practices to improve agility and mobility with the chair. Players need to be able to quickly maneuver around other players on the court and when they are on defense. If they cannot move the chair with accuracy, it can make performing skills during wheelchair basketball very difficult to perform.

Although this study shed new light on sport performance measures for amateur youth wheelchair basketball athletes, there were a few limitations. The sample size was only five players from a single team. Incorporating more youth wheelchair basketball teams could provide a more accurate depiction of the youth wheelchair basketball population. Due to time constraints of the athletes, we were not able to include cardiovascular endurance measures. Cardiovascular endurance is crucial to positive performance in wheelchair basketball. If players are not able to keep their aerobic endurance at high levels, the other athletes will have a distinct advantage. Also, all the players had the same disability, which can lead to similar data, as compared to having youth with different disabilities and performance levels. Using a population of youth players with different types of disabilities could lead to differentiations in flexibility/mobility and maybe more representative of other youth teams.

In future studies, including cardiovascular fitness by using the intermittent yo-yo test would show the cardiovascular endurance of each athlete. The yo-yo intermittent test involves each athlete sprinting between a measured distance with 10 seconds of active recovery between sprints until exhaustion is reached (Yanci et al, 2015). The time till exhaustion measures the amount of overall cardiovascular endurance. Researchers could also expand the population size and include various amateur teams to compare across amateur levels. It would be beneficial to perform a study which investigates the correlation between physical fitness levels and sport performance skill scores. Within the study, we were able to obtain valuable measurements to determine sport specific measures for amateur wheelchair basketball players. The data can be used to improve physical fitness areas and redesign practices to improve on areas that are important for success in youth wheelchair basketball.

References

1) BC Wheelchair Basketball Society, (2019). Wheelchair basketball skills: Beginner. Retrieved from http://bcwbs.ca/sites/default/files/users/documents/pdfs/Wheelchair_Basketball_Skills-Beginner.pdf.

2) Cavedon, V., Zancanaro, C., & Milanese, C. (2015). Physique and performance of young wheelchair basketball players in relation to classification. *PLoS ONE, 10(11)*, e0143621.

3) Coutts, K., (1992). Dynamics of wheelchair basketball. *Medicine and Science in Sports and Exercise*, *24*(2), 231–234.

4) Gil, S., Yanci, J., Otero, M., Olasagasti, J., Badiola, A., Bidaurrazaga-Letona, I., Iturricastillo, A., & Granados, C., (2015). The functional classification and field test performance in wheelchair basketball players. *Journal of Human Kinetics*, 46, 219-230.

5) Groot, S., Balvers, I., Kouwenhoven, S., & Janssen, T., (2012). Validity and reliability of tests determining performance-related components of wheelchair basketball. *Journal of Sports Sciences, 30(9),* 879-887.

6) International Wheelchair Basketball Federation, (2018). Classification. Retrieved from https://iwbf.org/the-game/classification/.

7) International Wheelchair Basketball Federation, (2019). Rules of Wheelchair Basketball. Retrieved from https://iwbf.org/rules-of-wheelchair-basketball/.

8) National Wheelchair Basketball Association, (2018). Junior division classification. Retrieved from https://www.nwba.org/juniorclassification.

9) Riebe, D., Ehrman, J. K., Liguori, G., & Magel, M. (2018). Health-Related Physical Fitness Testing and Interpretation. *ACSM's Guidelines for Exercise Testing and Prescription* (10th ed., pp. 66-110). China: Wolters Kluwer.

10) Tonson, A., Ratel, S., Le Fur, Y., Cozzone, P., Bendahan, D. (2008). Effect of maturation on the relationship between muscle size and force production. *Med Sci Sports Exercise* (40), 918–925.

11) Vanlandewijck, YC., Verellen, J., Beckman, E., Connick, M., &Tweedy, SM. (2011). Trunk strength effect on track wheelchair start: Implications for classification. *Medicine and Science in Sports and Exercise,* 43, 2344-2351.

12) Vanlandewijck, Y., Spaepen, A., & Lysens, R., (1995). Relationship between the level of physical impairment and sports performance in elite wheelchair basketball athletes. *Adapted Physical Activity Quarterly, 12,* (139-150).

13) What's Your Wingspan? (2019). Retrieved from http://sciencenetlinks.com/student-teacher-sheets/whats-your-wingspan/.

14) Yanci, C. Granados, M., Otero, A. Badioloa, J., Olasagasti, I. Bidaurranzaga-Letona, A. , Iturricastillo, & Gil, S. (2015). Sprint, agility, strength, and endurance capacity in wheelchair basketball players. *Biology of Sport, 32(1),* 71-78.

Evaluation of Varying Surface Finishes on Thin-Walled Blown Powder Deposition Inconel 625

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Abstract – Incorporation of additive manufacturing (AM) as another fabrication tool requires an understanding of the differences in material behavior from traditionally produced materials using casting, powder metallurgical or wrought processing. Since as-built AM parts typically have a rougher surface finish compared to parts subjected to traditional subtractive machining, post-processing methods to obtain a finer surface finish must be developed. This study evaluated the effect of various surface-finishing methods on additively manufactured Inconel 625. Blown powder deposition (BPD) was used to additively build a nominally 1 mm thick plate. After the build, specimens were subjected to: Chemically Accelerated Vibratory Finishing (CAVF), Chemical Milling (CM), and combinations of the two methods. Mechanical testing and microstructural characterization were used to evaluate the effect of the surface finish methods on the material. However, due to inhomogeneity of the as-built microstructure, no correlation could be made between the different surface finishes.

I. Introduction

Evaluation of additive manufacturing for replacing traditional subtractive machining must consider methods to post-process the as-built parts. While the ability to control tolerances and part dimensions depends on the AM process selected, the resulting surface finish can be modified by post-processing. Decreasing surface roughness is important as it has been correlated with reduced fatigue properties in AM materials such as Ti-6Al-4V [1]. This must be balanced with the decreasing number of grains in a cross section as the thickness of the part decreases [2]–[7]. With the potential for increased grain boundary area in a cross section, effects of chemical processing on the resulting microstructure and its correlation to material properties must be evaluated.

This study evaluated the effect of different surface finish methods on the resulting microstructure and mechanical properties of a blown powder deposition (BPD) AM of Inconel 625 built as a thin plate with nominal 1 mm thickness. The methods evaluated included: chemical milling (CM), chemically accelerated vibratory finish (CAVF), and combinations of the two. Applying chemical methods to a thin wall structure creates concern when using chemicals to improve the surface finish, and if they would attack the grain boundaries with subsequent detrimental effect on the mechanical behavior.

II. Experimental Procedure

BPD specimens were fabricated by (DM3D) in plates nominally 250 mm wide and 110 mm tall. No powder was provided for characterization, although the BPD process typically utilizes an average powder size of 100 mm. Specimens in the vertical orientation (build direction) and the horizontal orientation (build plane) were evaluated in this study as seen in **Figure 1**. This geometry differed from the ASTM E8 standard [9] for sub-sized tensile specimens with a narrower width and longer gauge length.

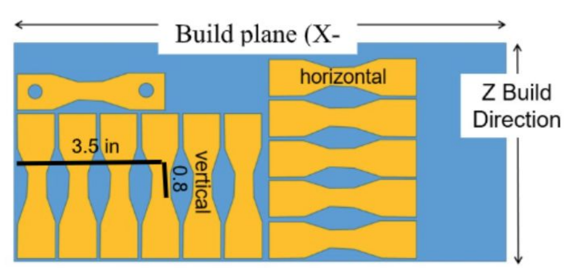

Figure 1. *Cut plan for dogbone specimens.*

After the build process, the specimens were homogenized at 1100 °C for 3 hours followed by an argon cool to 150 °C. Rem Surface Engineering subjected the dogbone specimens to their patented surface finishing process of CAVF in addition to a CM process and other combinations as summarized in **Table I**.

Surface Finish	**CAVF**	**CAVF/ CM**	**CM**	**CM/ CAVF**
Number of Specimens	3	3	2	3

Table I: *Number of Specimens and Surface Finishes*

The different surface finishes resulted in varying appearances of the specimens when observed underneath the microscope. To further analyze the differences in the surface finishes, both types were conducted to some of the specimens. There were two combinations, one consisted of the patented process followed by the standard chemical milling (CAVF/CM), and the other consisting of the standard chemical milling followed by the patented process (CM/CAVF). Each of the 11 Inconel 625 specimens were subjected to a uniaxial tension test. The tests were conducted according to ASTM E8 [9] with a constant crosshead velocity of 0.13 cm/min on a screw-driven load frame with a 250 kN load cell. Displacement was recorded as the cross-head movement. The load and displacement data were used to calculate the engineering stress vs strain data for each specimen. The yield strength (YS) was determined using the 0.2% offset strain method and the ultimate tensile strength (UTS) was based on the maximum load the specimen carried. After the tensile tests, specimens would be cut in the grip region for characterization of the microstructure in the build plane (horizontal) and the build direction (vertical). The specimens were mounted in a phenolic and prepared using standard metallographic procedures.

The as-polished cross-sections for each orientation were imaged to determine the void size and morphology. Images were obtained using a Zeiss inverted optical microscope with a 10x objective. The images were recorded at the interior of the specimen since there tends to be an increased number of voids at the edges in AM specimens. Sufficient images were recorded for the analysis of 1000 voids. ImageJ software [10] was utilized to analyze the void area. Void areas less than 0.2 mm were excluded as background noise. Due to expected variation in void size, the data was presented as boxplots.

After the void analysis, the specimens were etched using Aqua's Regia, a solution of nitric and hydrochloric acid in a mixed in 1:3 volume ratio, to reveal the grain boundaries. The grain structure was recorded over a range of objectives from 10x to 50x. A larger area of view was obtained by stitching images together which allowed analysis of the resulting wall thickness and surface finish. The different surface finishes resulted in a difference in the roughness of the specimens when observed underneath the microscope.

III. Results

Figure 2 shows the results of the tension testing and **Table II** summarizes these values. The CAVF/CM finish samples had the highest UTS while the CM finish had the lowest UTS. This same trend is also observed in the YS where the CAVF/CM specimens have the highest YS while the CM and CM/CAVF have the comparable lowest YS. Moreover, the same trends were observed in the elongation percentages. All values of the BPD additive manufactured specimens were lower than that of wrought Inconel 625.

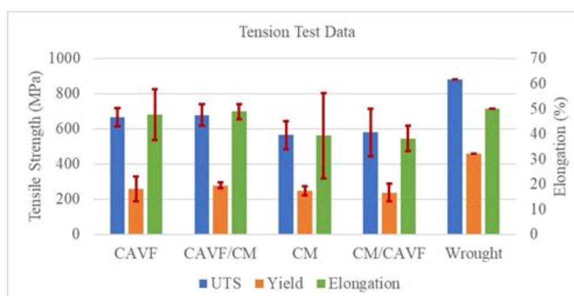

Figure 2. *Tension Property Comparisons*

On average, 1029 $\pm$ 59.00 voids were analyzed for the imaged samples. **Figures 3a and 3b** summarize the void area in the form of boxplots for the build plane and build direction, respectively. Overall, the void areas tend to be fairly uniform between the samples. **Figures 3c and 3d** summarize the void percentages with differences on the order of 0.1%.

A large variation was observed in the surface roughness between the processing methods. The CM and CAVF/CM specimens had rougher edges as compared to the other specimens that were smoother. This increased roughness can result in stress concentrations on the surface thereby affecting the mechanical properties, especially those of fatigue. **Figure 4** shows the cross-sectional views of the specimens to show surface roughness and **Table III** records the average thickness of each plane in the build direction along with standard deviations in thickness across the specimen.

Table II: *Tensile Data*

Surface Finish	As Built*	CAVF	CAVF/CM	CM	CM/CAVF	Wrought^
UTS (MPa)	646	666 ± 51	679 ± 61	565 ± 79	627 ± 136	880
Yield (MPa)	276	257 ± 71	278 ± 16	248 ± 25	285 ± 50.5	460
Elongation (%)	34.6	47.7 ± 10	48.9 ± 3	39.3 ± 17	38.1 ± 5	50.0

**One sample ("Special Metals Inconel@ Alloy 625")*

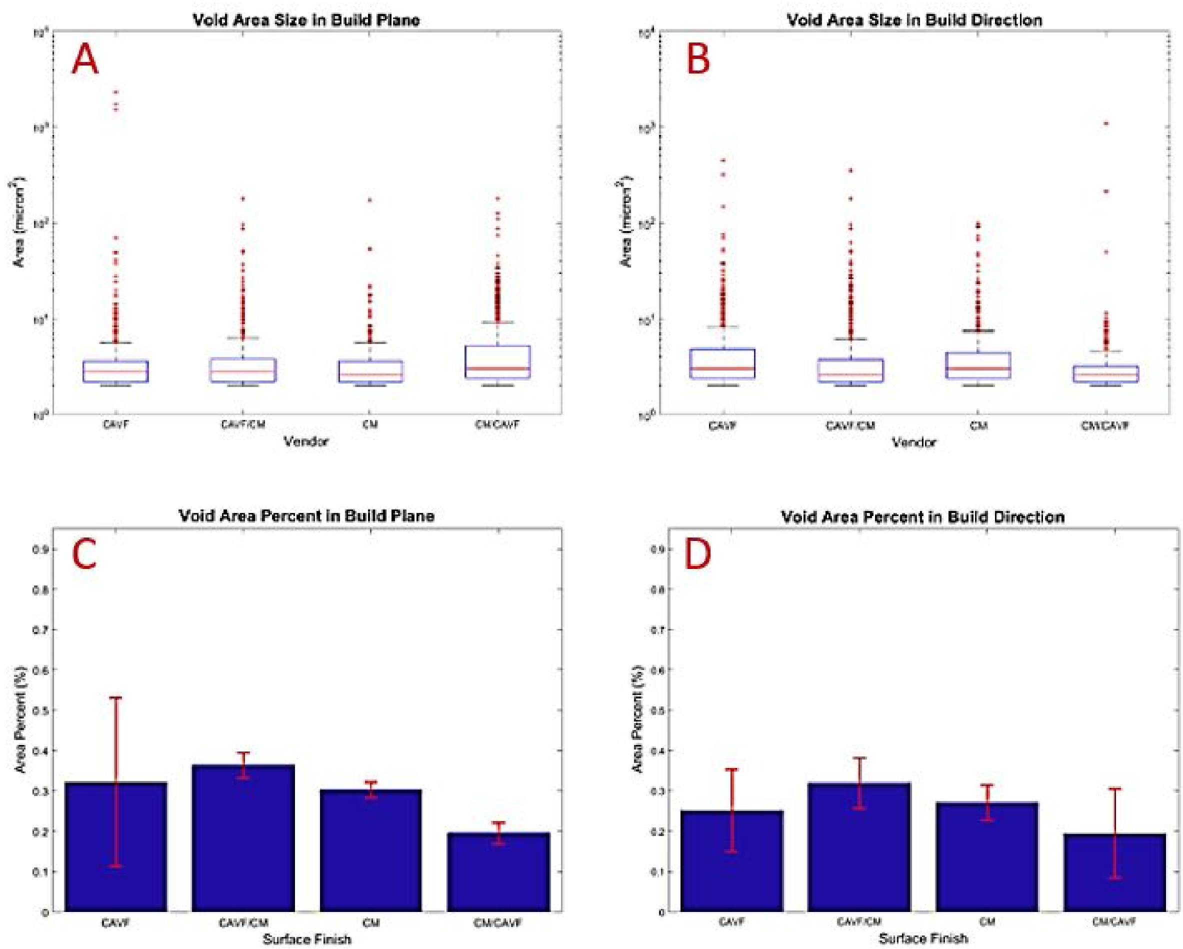

Figure 3. *Void Area for the* **(a)** *build plane and* **(b)** *build direction and void percentage for the* **(c)** *build plane and* **(d)** *build direction.*

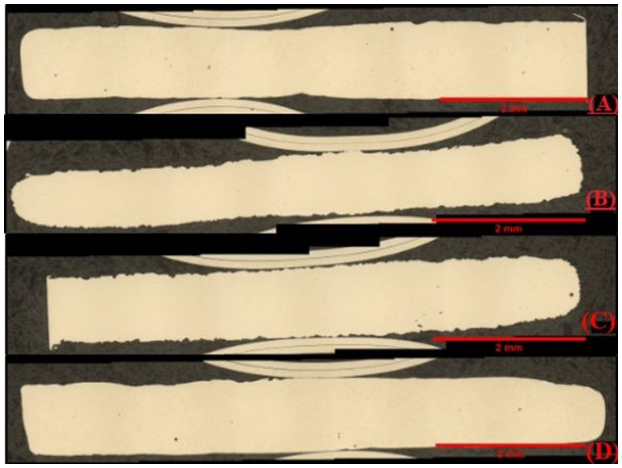

Figure 4. *Roughness comparison for the following samples:* **(A)** *CAVF,* **(B)** *CAVF/CM,* **(C)** *CM,* **(D)** *CM/CAVF*

Table III: *Thicknesses of Each Stitched Image in Build Direction*

Surface Finish Method	CAVF	CAVF/CM	CM	CM/CAVF
Average Thickness (µm)	909 ± 57	884 ± 39	802 ± 42	907 ± 74

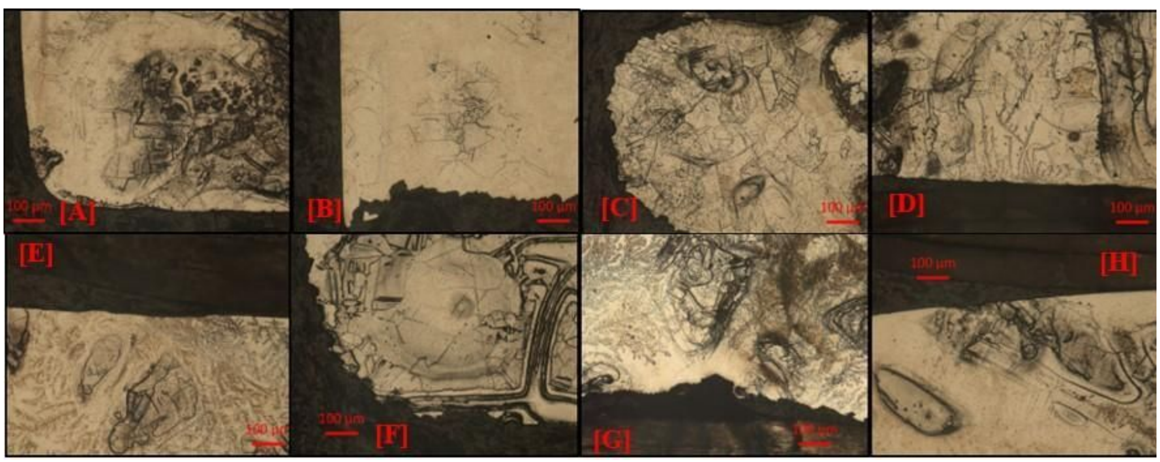

Figure 5. *Grain comparisons between specimen in Build Plane for* **(A)** *CAVF,* **(B)** *CAVF/CM,* **(C)** *CM, and* **(D)** *CM/CAVF and Build Direction for* **(E)** *CAVF,* **(F)** *CAVF/CM,* **(G)** *CM, and* **(H)** *CM/CAVF.*

In the build plane (vertical) images in **Figure 5**, dendritic structures were observed in the CM/CAVF (5D) specimen, while these structures were notably absent in the other specimens. These dendritic structures result from elemental segregation during solidification and are common in AM materials [4], [5]. More evidence of grain refinement was observed in the CM (5C) specimen. Interestingly, the CM specimen developed columnar grains near one edge of the build direction as shown in **Figure 5C**, resulting in a jagged end. This sort of grain formation was not found in other samples.

In the build direction, dendritic structures were observed in the CAVF and CM specimens, but not in the CAVF/CM and CM/CAVF specimens.

IV. Discussion

The CAVF/CM and CAVF specimens were about 100 MPa stronger than the CM, with the CM/CAVF specimens lower in strength by about 50 MPa. This supported the idea that CAVF increased the strength of the material but a more in-depth analysis on the grain structure was needed.

The void size and distribution were similar among the various specimens and not expected to explain the differences observed in the tensile strength.

The analysis of the etched samples indicated a wide variability in microstructural features in both the build direction and build plane. Although the specimens underwent a homogenization heat treatment, dendritic structures were still observed in the build direction of the CAVF and CM specimens.

Similar observations were made in the build plane microstructure as well, with dendritic formations only observed within the CM/CAVF specimen. Equiaxed grains were prominent within the CAVF/CM and CM specimens and less prominent in the build direction of the CAVF specimen. Although some equiaxed grains were observed within the CM/CAVF specimen, it was less common. Moreover, a large number of un-melted powders was observed in the CM specimen and not in other samples. This may explain why the tensile strength of the CM specimens were the lowest out of the four groups.

It is highly unlikely that the surface finish methods changed the interior microstructure of the material. It is more likely that the build process for the thin walled specimens did not produce a homogenous microstructure resulting in the variability observed. These microstructural variations would detrimentally affect the resulting mechanical properties [12]. As a result, the effect of the varying surface finishes on the thin walled Inconel 625 cannot be evaluated with the current lot of specimens.

Without consistent starting microstructures, the differences in strength based solely on the uniform surface finish cannot be evaluated. The CM/CAVF specimen had un-melted powders, indicated in **Figure 6**, that negatively affected the mechanical properties and there was dendrite formation throughout the different build planes in each of the specimen types. Several non-uniform grain structures were present in both build direction and build plane. To properly evaluate the correlation between surface finish and mechanical strength, more uniform build process is needed.

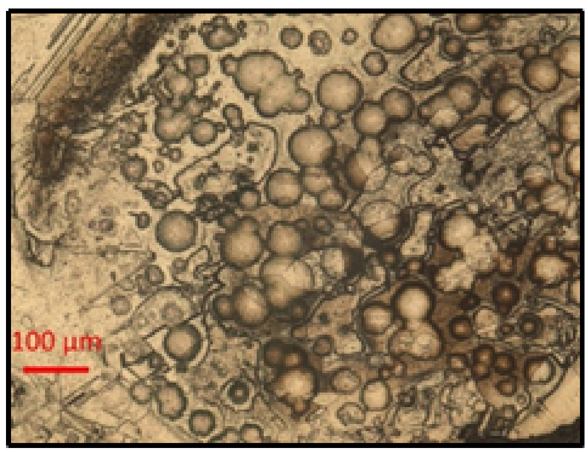

Figure 6. *Un-melted Powders within the Build Plane of the CM Specimen*

References

1) S. Bagehorn, J. Wehr, and H. J. Maier, “Application of mechanical surface finishing processes for roughness reduction and fatigue improvement of additively manufactured Ti-6Al-4V parts,” *Int. J. Fatigue*, vol. 102, pp. 135–142, 2017.

2) M. Fullen and J. Schneider, “Effects of varying heat treatments on the microstructure and mechanical properties of blown powder Inconel 625,” pp. 1–9.

3) F. Zhang *et al.*, “Effect of heat treatment on the microstructural evolution of a nickel-based superalloy additive-manufactured by laser powder bed fusion,” *Acta Mater.*, 2018.

4) F. Lia, J. Z. Park, J. S. Keist, S. Joshi, and R. P. Martukanitz, “Thermal and microstructural analysis of laser-based directed energy deposition for Ti-6Al-4V and Inconel 625 deposits,” *Mater. Sci. Eng. A*, vol. 717, no. September 2017, pp. 1–10, 2018.

5) G. P. Dinda, A. K. Dasgupta, and J. Mazumder, “Laser aided direct metal deposition of Inconel 625 superalloy: Microstructural evolution and thermal stability,” *Mater. Sci. Eng. A*, vol. 509, no. 1–2, pp. 98–104, 2009.

6) S. Li, Q. Wei, Y. Shi, C. K. Chua, Z. Zhu, and D. Zhang, “Microstructure Characteristics of Inconel 625 Superalloy Manufactured by Selective Laser Melting,” *J. Mater. Sci. Technol.*, 2015.

7) M. LIU, W. jie ZHENG, J. zhong XIANG, Z. gang SONG, E. xiang PU, and H. FENG, “Grain Growth Behavior of Inconel 625 Superalloy,” *J. Iron Steel Res. Int.*, 2016.

8) DM3D, “DM3D Technology.” [Online]. Available: http://www.dm3dtech.com/index.php?option=com_content&view=featured&Itemid=435.

9) ASTM Int., “Standard Test Methods for Tension Testing of Metallic Materials 1 - ASTM E8M-13a,” *Astm*, no. C, pp. 1–28, 2014.

10) W. S. Rasband, “ImageJ,” *National Institutes of Health*.

11) “Special Metals Inconel@ Alloy 625,” *Matweb*. [Online]. Available: http://www.matweb.com/search/datasheet.aspx?MatGUID=4a194f59f35a427dbc5009f043349cb5.

12) J. T. Black and R. A. Kohser, “Nature of Metals and Alloys,” in *Materials and Processes in Manufacturing*, Tenth., Wiley, 2007, p. 56.

Effects of Homogenization Heat Treatment on the Microstructure and Mechanical Properties of Blown Powder Deposition Inconel 625 Specimens

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Abstract **–** Additive manufacturing (AM) is a relatively new process for directly building metallic components in a layer-by-layer manner. This can reduce fabrication costs in alloys that are difficult to subtractively machine, especially for low volume, complex components. Due to the rapid solidification nature of the process, elemental segregation is a concern as it can detrimentally affect the resulting mechanical properties. Thus, post-processing homogenization heat treatments are applied to ensure effectiveness of the alloying system and obtain isotropic properties. Standards to guide post-processing of AM materials are still under development; thus, more information is needed on how the variability among vendors affects the resulting mechanical properties. This study evaluates the effectiveness of a homogenization heat treatment on the mechanical properties and microstructure of Blown Powder Deposition (BPD) Inconel 625 produced by four different vendors.

I. Introduction

Industrial usage of metals relies on alloying to improve the mechanical strength while retaining the physical properties of the base metal. Nickel (Ni) based superalloys are used in applications where retention of mechanical strength at elevated temperature and resistance to thermal creep, corrosion, and oxidation is required. Inconel 625 is a solid solution strengthened, Ni-based superalloy, which is used in various applications, from maritime to aerospace. However, due to its high strength and rapid work hardening, Inconel is a difficult material to subtractively machine. This makes it an attractive alloy for AM processing. Additionally, as aerospace applications typically require the production of low volume, complex components, AM has the potential to reduce processing time and costs since components can be built as one piece. However, as AM fabrication of metal alloys is relatively new, standards are needed to ensure consistency in the resulting microstructure and mechanical properties.

In AM deposition, the metal deposited undergoes rapid and repeated localized melting, solidification and reheating. Similar to castings, shrinkage voids and elemental segregation detrimentally affect the resulting mechanical properties. In order to alleviate the mechanical anisotropic behaviors of AM Inconel 625, a homogenization heat treatment process commonly used in castings, can be applied [1]. During the rapid solidification of alloys, elemental segregation occurs forming structures called dendrites due to their branch-like formation [2]. Typically, the dendrites are driven back into solution in a post processing homogenizing heat treatment. The time and temperature of the homogenization process relates to the spacing, or diffusion distance, between the branches. Since homogenization is a diffusion process, less time is required at higher temperatures. However, other constraints may influence the selected temperature range such as assembly and subsequent processing.

ASTM B443 has laid out the standards for Inconel 625 wrought properties and heat treatment and recommends that wrought Inconel 625 be annealed at a minimum of 1093ºC [3]. In previous work on BPD Inconel 625, Fullen et al. [4] found differences in the resulting microstructure of specimens obtained from four different vendors after various homogenization schedules. The highest degree of homogenization was reported after heat treatment at 1150 °C for 3 hours [4]. Since fabricated components will likely come from different vendors, this study focused on understanding the variation in mechanical properties if the homogenization heat treatment parameters of time and temperature were standardized. To evaluate the resulting mechanical properties, tensile and hardness tests were conducted on the specimens after a common homogenization heat treatment. Heat treated specimens were metallurgically evaluated using optical microscopy to document their microstructure.

II. Experimental Procedure

Single pass, 3mm thick plates of Inconel 625 were obtained from four vendors using BPD AM. The plates were nominally 250 mm wide by 110 mm tall. **Figure 1** illustrates the geometry of the tensile bars cut from the flat plates using Electron Discharge Machining (EDM). **Figure 2** shows the orientation of the specimens, six for each vendor (three along build plane and three along build direction) for a total of 24 specimens. After tension testing, specimens were removed from the grip sections for metallurgical preparation of the build direction (Z) and build plane (XY) surfaces, shown respectively in blue and green.

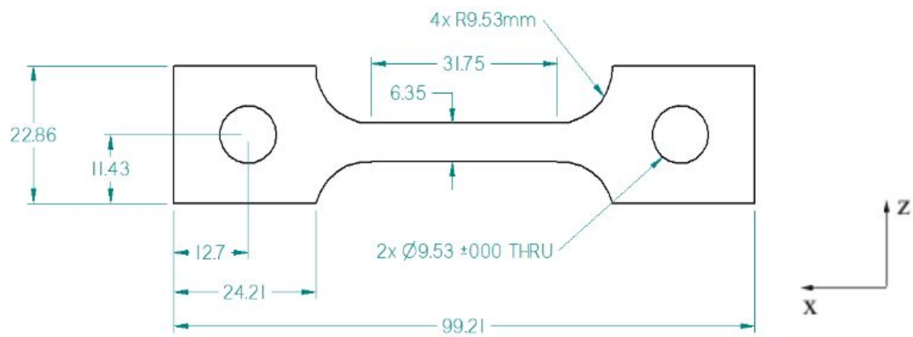

Figure 1. *Sub-size Tensile Geometry (all dimensions in mm)*

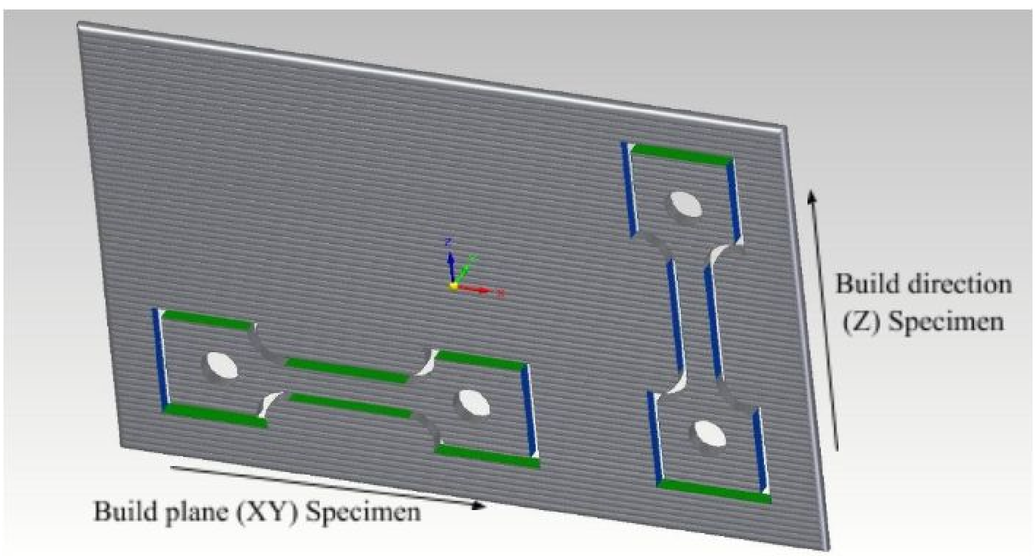

Figure 2. *Nominal plate showing orientation of the build direction and build plane specimens.*

Homogenization heat treatment was done using a Cone Art Kilns BX199D with Bartlett Instruments Co. V6-CF control panel. The specimens were placed in a preheated kiln operated in an air environment. To prevent oxidation during the heat treatment, the specimens were wrapped in stainless steel foil that was removed prior to tensile testing. **Table 1** lists the heat treatment parameters. After each heat treatment, the specimens were removed from the furnace and air cooled.

Heat Treatment	Temperature (°C)	Time (hr)
Stress Relief	900	1
Homogenization	1150	3

Table 1. *Heat Treatment Parameters*

All tensile tests were conducted on an Instron® 5985 electromechanical load frame. Stress was calculated based on the loads obtained from a 250kN load cell and individual specimen dimension measurements. The specimens were tested at a constant crosshead velocity of 0.13 cm/min in accordance with ASTM E8 [5].

After tensile testing, a section from the grip region was abrasively cut to reveal the build plane (XY) and build direction (Z) internal surfaces. The cut samples were mounted in a phenolic and were metallographically prepared including a final polish of 0.5µm Alumina powder (Al2O). A Zeiss™ Axio Vert.A1 Inverted Microscope for Reflected Light Techniques was utilized to capture images after polishing and etching using Aqua Regia [7].

Hardness tests were conducted using a Wilson Hardness Tester on the Rockwell B scale. Average values were recorded from 4 indentations per specimen per orientation. The hardness values were used to evaluate the homogeneity of the resulting microstructure and compare with the data from tensile testing.

III. Results and Discussion

The average tensile data of build direction (Z) and build plane (XY) specimens for the four vendors are presented in **Figure 3a and 3b**, respectively. Markers are added to **Figure 3** to present the wrought properties [3]. The results of the as-built samples agree with literature on AM specimens which show a reduction in strength of specimens tested in the build direction as compared with the build plane [4]. After homogenization heat treatment, the average data between build plane and build direction specimens are nearly identical for vendor 1 and 3, while vendor 4 has a slight decrease and vendor 2 has a significant drop in Ultimate Tensile Strength (UTS).

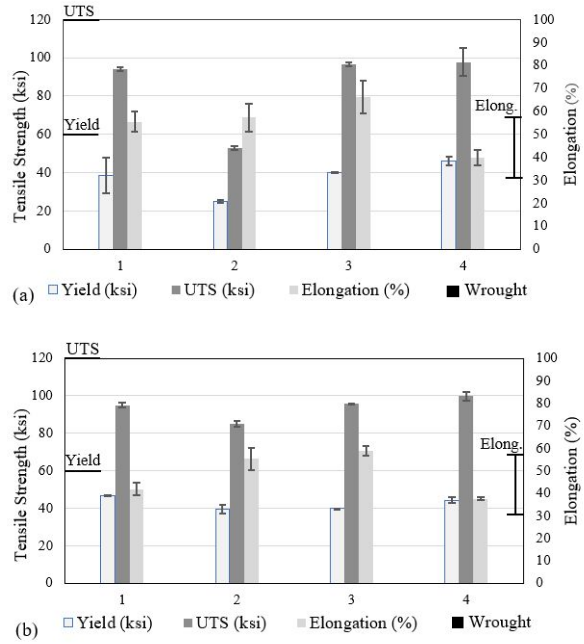

Figure 3. *Average of the mechanical properties of* **(a)** *build direction specimens and* **(b)** *build plane specimens.*

The average hardness testing results are summarized in **Figure 4**. The hardness results agree with the tensile data shown in **Figure 3**. The hardness of both build direction and build plane specimens is comparable for vendor 1 and 3. For vendor 2 and 4, the hardness, on average, is lower in the build direction orientation. These results match the tensile test results, which show that vendor 2's strength is significant lower post heat treatment in build direction specimens.

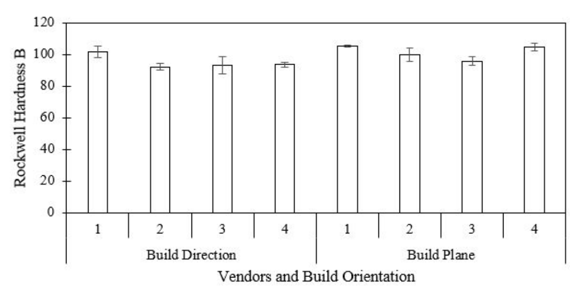

Figure 4. *Hardness Testing Results*

After homogenization heat treatment, there were two predominant patterns found in the specimens as illustrated by the representative images in **Figures 5 and 6** for vendors 3 and 4, respectively. Only these images are shown as specimens from vendors 1 and 4 responded in a similar fashion as did specimens from vendors 2 and 3. Post heat treatment, specimens from vendors 2 and 3 showed evidence of grain definition as can be seen in **Figure 5a and 5b** for the two orientations. Higher magnification images in **Figure 5c and 5d** show slight remains of the former dendritic structure. Arrows indicate grain boundaries at the higher magnification.

In contrast, for **Figure 6a and 6b**, the grains are much larger, and the grain boundaries are not well defined. A dendritic pattern is especially apparent in the higher magnification images specimens in **Figure 6c and 6d**. The dendrites form as cellular structures that are viewed end on in the **Figure 6c** and along their length in **Figure 6d**. It is interesting to note that degree of homogenization and recrystallization appear to have a connection in this study.

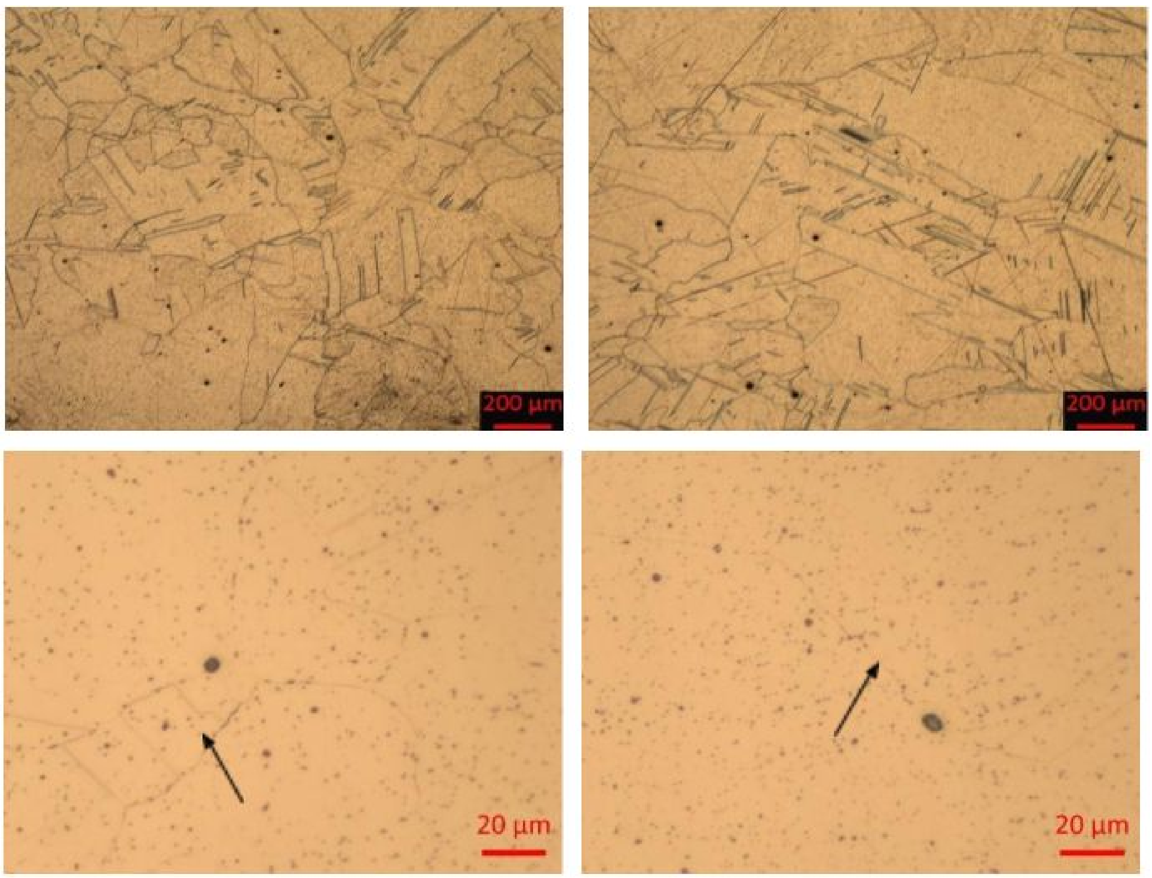

Figure 5. *Vendor 3 build plane specimen at 5x magnification* **(a)** *build plane (XY) and* **(b)** *build direction (Z) surfaces, and at 50x magnification* **(c)** *build plane (XY) and* **(d)** *build direction (Z) surfaces.*

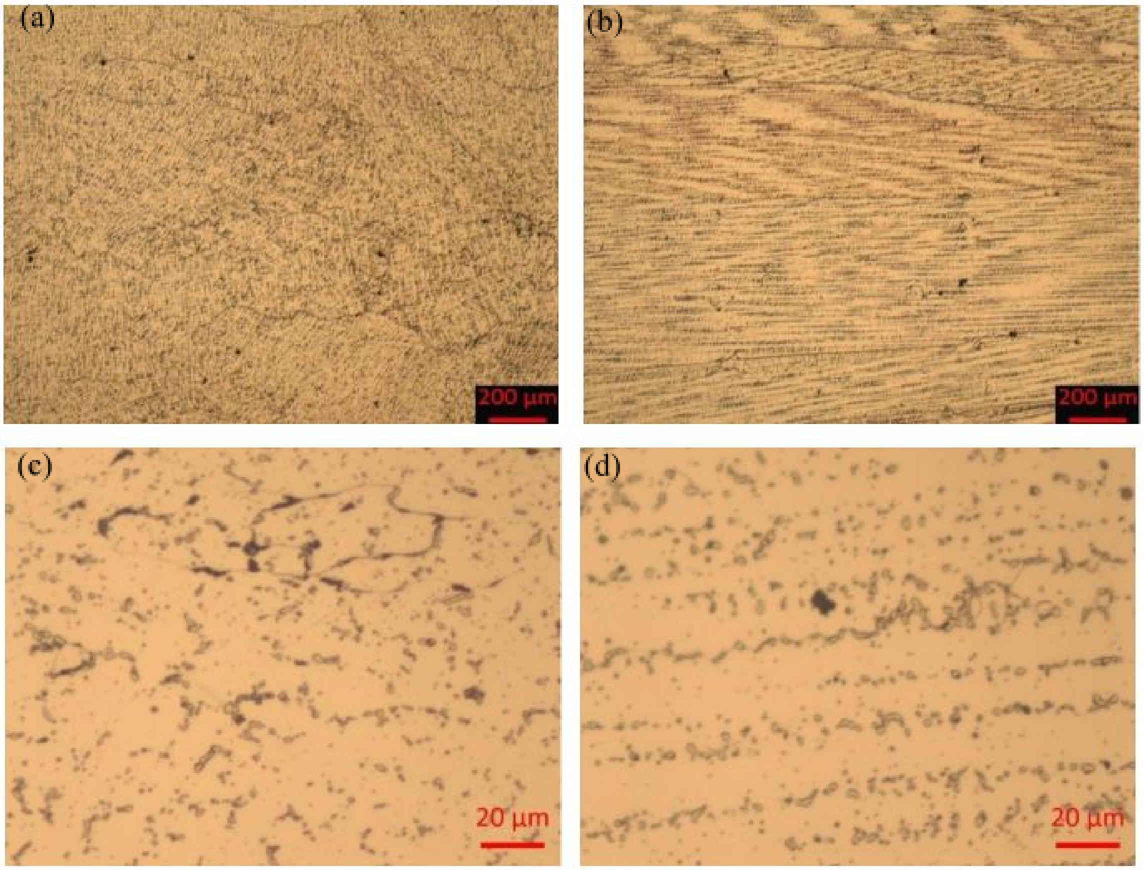

Figure 6. *Vendor 4 build plane specimen at 5x magnification* **(a)** *build plane (XY) and* **(b)** *build direction (Z) surfaces, and at 50x magnification* **(c)** *build plane (XY) and* **(d)** *build direction (Z) surfaces.*

IV. Summary

BPD AM is an attractive alternative manufacturing method for low volume and complex Inconel 625 components for applications that require high strength, corrosion resistance, and thermal resistance. Since it was observed that as built BPD AM Inconel 625 parts have heterogeneous microstructures, it was necessary to investigate the effect of post processing methods such as homogenization heat treatment. This study has found that after a heat treatment at 1150ºC for 3 hours, variation was observed in the degree of homogenization obtained in the samples that correlated with the degree of recrystallization. Except for vendor 2, the mechanical properties of tensile strength and hardness were improved after homogenization. The variation in mechanical properties and microstructure could correlate with either the vendors' equipment, processing parameters or starting feedstock. As a result, it is recommended that the development of standards for AM processing considers these aspects.

V. Acknowledgements

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References

[1] P. D. Jablonski, and J. A. Hawk. “Homogenizing Advanced Alloys: Thermodynamic and Kinetic Simulations Followed by Experimental Results.” Volume 26, Issue 1, pp 4–13, 2017.

[2] H. L. Wei, T. Mukherjee, and T. DebRoy. “Grain Growth Modeling for Additive Manufacturing of Nickel Based Superalloys.” Proceedings of the 6th International Conference on Recrystallization and Grain Growth (ReX&GG 2016), pp 265-269, 2016.

[3] ASTM B443, Standard Specification for Nickel-Chromium-Molybdenum-Columbium Alloy (UNS N06625) and Nickel-Chromium-Molybdenum-Silicon Alloy (UNS N06219) Plate, Sheet, and Strip. ASTM International, West Conshohocken, PA (2014).

[4] M. Fullen, and J.A. Schneider. “Effects of varying heat treatments on the microstructure and mechanical properties of blown powder Inconel 625.” JOM, Volume 71, Issue 3, pp 1127–1133, 2019.

[5] ASTM E8/E8M, Standard Test Methods for Tension Testing of Metallic Materials. ASTM International, West Conshohocken, PA (2016).

[6] W.S. Rasband. ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, https://imagej.nih.gov/ij/, (1997-2018).

[7] Compound Summary of Aqua Regia. PubChem, National Center for Biotechnology Information.

Thermococcus thioreducens' Inorganic Pyrophosphatase Purification

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***Abstract* –** Protein purification requires an intensive multi-step process that is customized to every protein's characteristic for optimal results. In this study, we looked at the purification process of Inorganic Pyrophosphatase (IPPase), an essential protein found in all living organisms. The commonality of the protein does not veil the fact that the mechanism by which IPPase is hydrolyzed into two orthophosphates is not fully understood. To comprehend the mechanism, recombinant mutants of the conservative region in IPPase were expressed. However, to observe a possible change in structure of the recombinant mutants with crystallization, pure concentrated protein is needed. Therefore, protein purification is crucial. During this purification process, three characteristics of IPPase are manipulated to create an acceptable purification: IPPase's thermophilic nature, its isoelectric point, and the 21 kDa size of the monomer. With the perfection of functioning protocol, steps can begin to be taken in the studying of the activity of each integral amino acid.

I. Introduction

Inorganic Pyrophosphatase (IPPase) is a catalytic enzyme responsible for the hydrolysis of pyrophosphate (PP_i), a byproduct of DNA and RNA synthesis, into two orthophosphate (P_i). IPPase is an integral protein to all living organisms that upon its activation pushes the reaction equilibrium towards synthesis of nucleic acids [4]. The cofactor used in the mechanism by which the substrate is hydrolyzed divides the enzyme species into two families, family I ,which involves coordination via a Mg^{2+} ion, and family II, which involves a Mn^{2+} ion. Both serve a similar function, by oxidizing an incoming water molecule, the H_2O will then become nucleophilic and attack the electrophilic phosphate, thus lysing the PP_i into its two separate P_i molecules. However, although both species have similar active sites and have similar mechanisms, the rest of the protein sequence is nonhomologous. Family I is the most common and well-studied being found in organisms that include archaea, prokaryotes, and eukaryotes while family II is commonly found in human infectious prokarya.

Thermococcus thioreducens is a hyperthermophilic archaea bacteria found in black chimney residue of Rainbow deep sea vents in the Mid Atlantic Ridge [2]. *Thermococcus thioreducens* consists of a circular coccoid body with a singular flagellum for motility. Effective growth for this archaic bacterium is within a pH range of pH 5.0- pH 8.5, optimally pH 7.5, a salt concentration of 1%-5%, optimally 3%, and a temperature of ℃ 55 - 94, optimally ℃ 83 - 86. Growth is strictly anaerobic and requires elemental sulfur as its electron acceptor. This species of IPPase is of interest due to its chemical stability and thermostability; due to this stability it can be used in long duration studies. It is a family I IPPase with a 21 kDa molecular mass.

To purify IPPase from a cell, the intrinsic properties of the enzyme must be taken into consideration. *Thermococcus thioreducens* IPPase (Tt IPPase) is a very thermostable enzyme, where other enzymes would denature at high temperatures, such as 72 ℃, Tt IPPase stays in solution and does not denature; therefore, a heat cut is a viable first step for removing purifying Tt IPPase [1]. Tt IPPase's isoelectric point is also known to be roughly pH 5; therefore, when the protein is put into a buffer at a higher pH, like pH 7.5, the protein will be negatively charged and thus susceptible to an anion exchange chromatography. An anion exchange chromatography column utilizes negative charge of the protein to separate out the proteins in solution, a rising ion concentration outcompetes weaker charged anions and thus they will elute off while more strongly bound negative proteins will take a higher ion concentration to elute off [3]. Tt IPPases size is also known to be roughly 21 kDa as a monomer and therefore a size exclusion chromatography column will separate out any further unwanted proteins. Once the crude cell lysate has gone through these purifications a pure sample of Tt IPPase will be left for a protein assay and X-ray crystallography to determine concentration and 3D structure respectively. Isolation of Tt IPPase monomers has been inconsistent in previous attempts outlining the need for a streamlined and reproducible method; one that effectively purifies the Tt IPPase for X-ray crystallography, snapshotting the mechanism by which IPPase is hydrolyzed.

II. Materials and Methods

Transformation

Firstly, a plasmid that contains both antibiotic resistance against chloramphenicol and carbenicillin as well as the DNA sequencing for *Thermococcus thioreducens* IPPase was added to a solution of competent *E. coli* cells. The DNA sequence also contained the antibiotic resistance that ensured growth of the expressing bacteria. The cells are kept in a -80 ℃ freezer and must thaw over ice for one-time use. The DNA sequence was added in a 1:25 volume: volume ratio with the cells and allowed to incubate on ice for 20 minutes. Once these cells were incubated, they were placed in a 42 ℃ heat shock to activate the cells for DNA acceptance. Then the cells were suspended in 1 mL LB Miller broth, which would supply the nutrients necessary for the cells' growth. This growth takes place in a 37 ℃-water bath for 45 minutes. This now turbid cell solution had 1 mL poured over an agar plate that contained 50 µg/mL carbenicillin and 30mg/mL chloramphenicol. Cells were then allowed to grow over night on the plates.

Small-Scale Inoculation

Upon formation of cell colonies, production of IPPase can be screened for. Half of one cell colony on the plate was inoculated into 25 mL LB Miller media with the same antibiotic concentration as the agar plate. Once the cells were grown, the cells were lysed via sonication, and transferred to a microtube. Then protein was roughly purified through heat cut, a process where the cell solution is placed in a 72℃ water bath and denatured proteins coagulate in the bottom of the microtube leaving a solution of thermostable proteins including Tt IPPase. Protein expression of the different colonies were portrayed through an SDS-PAGE. When overexpression of IPPase at the 21 kDa range was present, the other half of the colony would be used for a large scale inoculation.

Large-Scale Inoculation

Half of the colonies were inoculated each into 125 mL Erlenmeyer flasks containing 50 mL of sterilized LB miller broth, 50 mg/mL chloramphenicol, and 100 mg/mL carbenicillin. The flasks were left to incubate at 37 ℃ in a shaker overnight. After 21 hours, 10 mL of the now turbid solution is transferred to a 4000 mL volumetric flask containing 1000 mL of sterilized LB miller, 50 mg/mL chloramphenicol, and 100 mg/mL carbenicillin. The flasks were left to be incubated at 37 °C in a shaker. The absorbance was checked at every hour at 600 nm to determine the moment before the bacteria start their exponential growth, estimated to be at an absorbance of 0.300. After 3 hours and 20 minutes, bacteria solution reached desired absorbance. At this time, the temperature was dropped to 30 ℃, and IPTG was added for an overall concentration of 0.5 mM to induce activation of the lac operon. The solution was left to shake overnight.

Lysing Bacteria

The turbid solution was transferred into four 250 mL centrifuge bottles and balanced. The solution was centrifuged down at 6000 rcf at 25 ℃ for 7 minutes. After centrifuging, bacterial pellets were collected and weighed into no more than 10 g pellets and plastic wrapped. The 10g mass was found to be the optimal size for protein concentration while not overloading a 40 mL anion exchange column. The pellet was stored at -80 ℃ overnight. The pellet was unwrapped frozen and resuspended into 50 mL lysis buffer of 25mM NaCl, 2 mM EDTA, 1 mM PMSF, 0.1 mg/mL lysozyme, and 50 mM Sodium Phosphate pH 7.5. Once resuspended, the bacteria were further lysed using sonication at 5 minutes of on and off cycles until solution is homogeneous. The sonicated solution was transferred into round bottom centrifuge tubes and centrifuged for 1 hour at 4 ℃ and 7000 rcf. The supernatant was collected, and the cell debris pellet disposed.

Heat Cut

The supernatant was transferred into a 125 mL volumetric flask, where DNase stock solution was added at 500 µL per 100 mL. To activate the DNase, 5 mM $MgCl_2$ and 1 mM $CaCl_2$ was added to the solution and allowed to incubate at room temperature for 30 minutes. This breaks down DNA contaminant into smaller strands for easier removal from the solution. Afterwards, the flask was placed in a 72 ℃ water bath for 45 minutes. The heat cut will denature any proteins not stable at 72 ℃, which will then coagulate together and form a pellet in the microtube after centrifugation. The heat cut solution is transferred into round bottom centrifuge tubes and centrifuged for 1 hour at 4 ℃ and at 7000 rcf. The supernatant is collected into conical tubes and the pellet disposed.

Anion Exchange Column

A 20 mL Q-sepharose column was charged with a resolving buffer consisting of 25 mM Sodium Phosphate buffer pH 7.5, 25 mM NaCl, 1 mM PMSF, and 1 mM EDTA. Once the column was charged the protein sample was loaded onto the column and a linear gradient buffer switch was started. This buffer switch consists of a change from the aforementioned resolving buffer to an identical 1 M NaCl concentration elution buffer. During this linear gradient, fractions of a 10 mL volume were collected and tested in a Nanodrop spectrophotometer as well as an SDS-PAGE for protein concentration. These tested fractions would later be combined and concentrated for size exclusion chromatography.

Size Exclusion

The samples were concentrated down by spinning down the solution in a 10 kDa concentrator tube that is spun down in a centrifuge at 4 ℃, 6000 rcf for 20 minutes. The protein sample was concentrated down to a volume of 500 µL before being carefully pipetted evenly onto a 120 mL S100 size exclusion column. A 50 mM HEPES 25 mM NaCl buffer was run through the column to both charge it before the sample was loaded, and then it was run with the sample, both at 1 mL/min. Once the concentrated sample was loaded onto the column 10 mL fractions were collected and tested on the Nanodrop for protein concentration.

III. Results

In an SDS-PAGE, protein is denatured and run through the gel. Tt IPPase is found in the 21 kDa region, but as seen in **Figure 1** there were other proteins present throughout the gel.

With the peaks being cut off due to machine limitations in **Figure 2**, Nanodrop readings and SDS-PAGE of each fraction was necessary for determining which fractions would be conserved for size exclusion. A high A280/260 ratio would signify high protein presence. A280 refers to absorbance of UV light that is emitted by tryptophan amino acids at a wavelength of 280 nm, while A260 refers to an absorbance due to nucleotides. Those with high IPPase (21 kDa) expression were concentrated down for size exclusion gel filtration.

An S100 chromatography column, as seen in **Figure 3**, was used to separate proteins based on weight up to 100 kDa, allowing larger proteins to flow through and be collected in earlier fractions, while smaller proteins would pe trapped in the gel and take longer to elute off. Nanodrop and SDS-PAGE of the fractions determined where the IPPase had eluted off.

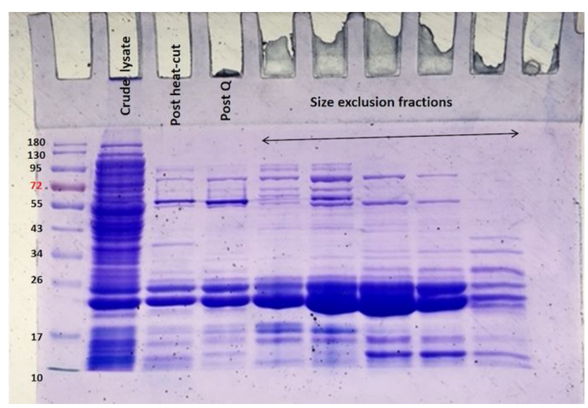

Figure 1. *SDS - PAGE for D68N containing protein expression for Crude, Post-Heat Cut, Anion Exchange, and Size Exclusion.*

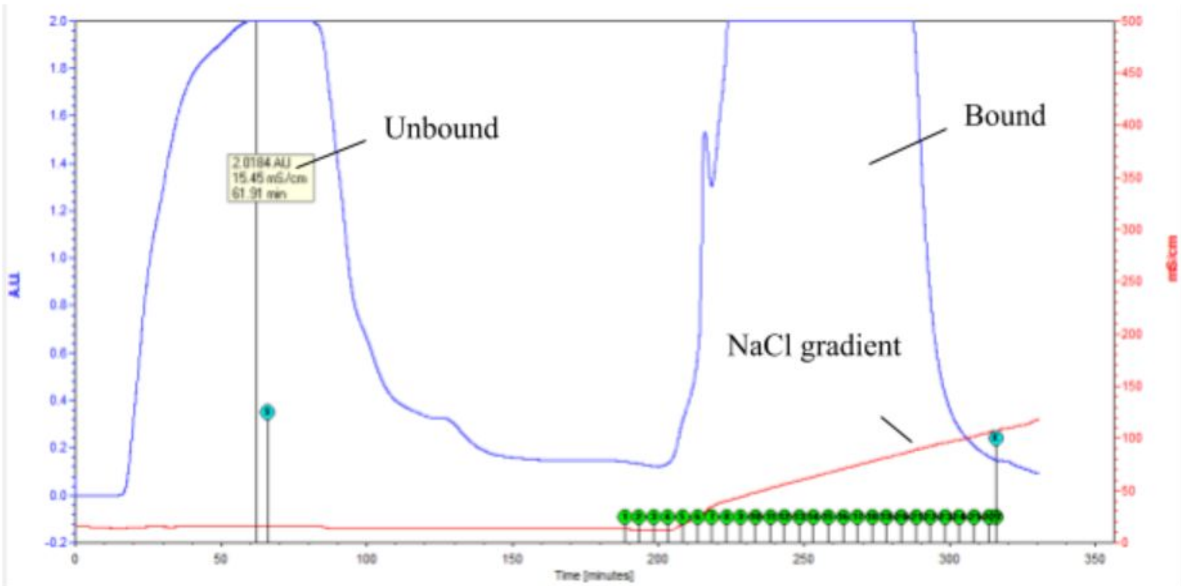

Figure 2. *Anion Exchange Column graph, which contains the unbound protein and the bound protein. The blue line is 280 am wavelength absorption while the red line is salt concentration.*

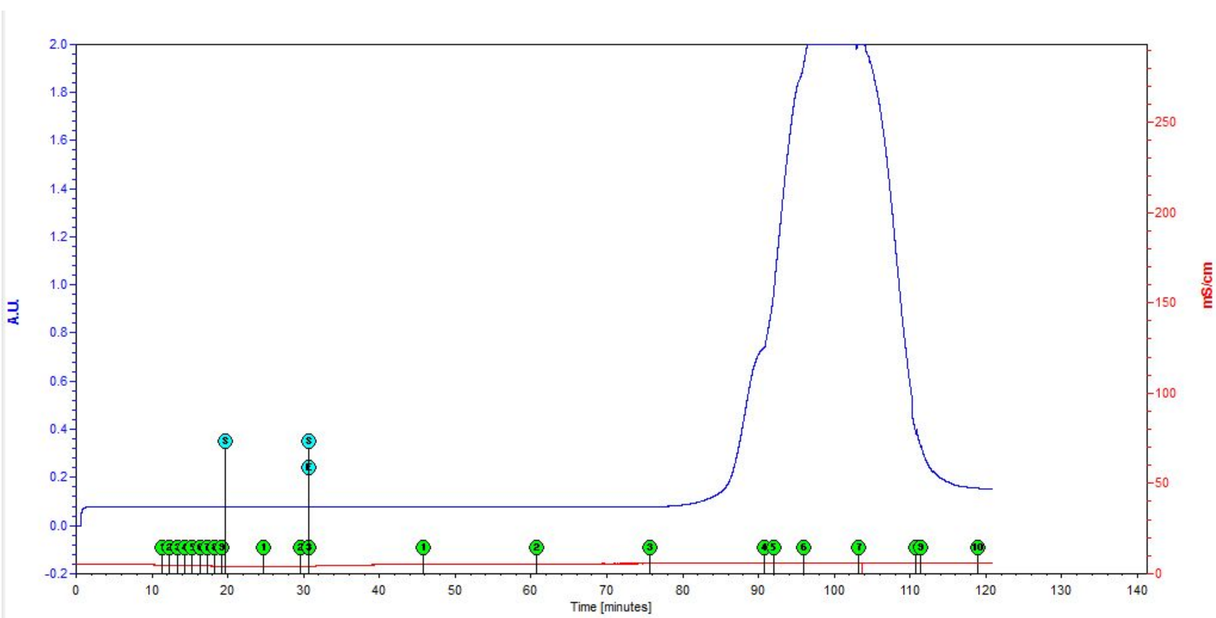

Figure 3. *Exclusion graph. The blue line is 280 am wavelength absorption while the red line is salt concentration.*

V. Conclusion

As seen in **Figure 1**, the protein purity had not increased as expected between the anion exchange and size exclusion chromatography. There was still expression of smaller proteins in the fractions that also contained the Tt IPPase expression. The solution moving forward is to change the anion exchange column volume from 20 mL to 40 mL to allow more surface area for Tt IPPase to bind. Another change in protocol for the anion exchange is running the column over a longer duration of a linear salt gradient to ensure better elution and small peaks in the chromatography. Once the second anion exchange is completed, the fractions containing IPPase can then be sent through size exclusion yet again to yield pure protein. With the size exclusion data seen in **Figure 3**, the peaks were unable to be accurately observed due to being off chart. To fix the data, the size exclusion should run at a slower flow rate than the previous 1 mL/min to allow a better separation of peaks.

Overall, Tt IPPase was present throughout every set of the protein purification, so the characteristics of the protein were accurately manipulated. Unfortunately, Tt IPPase was not the only protein present, which prevented any crystallization and would interfere with future protein assay.

References

1) Hughes, R., Coates, L., Blakeley, M., Tomanicek, S., Langan, P., Kovalevsky, A., … Ng, J. (2012). Inorganic pyrophosphatase crystals from Thermococcus thioreducens for X-ray and neutron diffraction. *Acta Crystallographica. Section F: Structural Biology and Crystallization Communications*, *68*(12), 1482–1487. https://doi.org/10.1107/S1744309112032447.

2) Pikuta, E.V., Marsic, D., Itoh, T., Bej, A.K., Tang, J., Whitman, W.B., Ng, J.D., Garriott, O.K., & Hoover, R.B. (2007). Thermococcus thioreducens sp. nov., a novel hyperthermophilic, obligately sulfur-reducing archaeon from a deep-sea hydrothermal vent. International journal of systematic and evolutionary microbiology, 57 Pt 7, 1612-8.

3) Structural Genomics Consortium, China Structural Genomics Consortium, Northeast Structural Genomics Consortium, Gräslund, S., Nordlund, P., Weigelt, J., Hallberg, B. M., Bray, J., Gileadi, O., Knapp, S., Oppermann, U., Arrowsmith, C., Hui, R., Ming, J., dhe-Paganon, S., Park, H. W., Savchenko, A., Yee, A., Edwards, A., Vincentelli, R., Cambillau, C., Kim, R., Kim, S. H., Rao, Z., Shi, Y., Terwilliger, T. C., Kim, C. Y., Hung, L. W., Waldo, G. S., Peleg, Y., Albeck, S., Unger, T., Dym, O., Prilusky, J., Sussman, J. L., Stevens, R. C., Lesley, S. A., Wilson, I. A., Joachimiak, A., Collart, F., Dementieva, I., Donnelly, M. I., Eschenfeldt, W. H., Kim, Y., Stols, L., Wu, R., Zhou, M., Burley, S. K., Emtage, J. S., Sauder, J. M., Thompson, D., Bain, K., Luz, J., Gheyi, T., Zhang, F., Atwell, S., Almo, S. C., Bonanno, J. B., Fiser, A., Swaminathan, S., Studier, F. W., Chance, M. R., Sali, A., Acton, T. B., Xiao, R., Zhao, L., Ma, L. C., Hunt, J. F., Tong, L., Cunningham, K., Inouye, M., Anderson, S., Janjua, H., Shastry, R., Ho, C. K., Wang, D., Wang, H., Jiang, M., Montelione, G. T., Stuart, D. I., Owens, R. J., Daenke, S., Schütz, A., Heinemann, U., Yokoyama, S., Büssow, K., … Gunsalus, K. C. (2008). Protein production and purification. *Nature methods*, *5*(2), 135-46.

4) Structural Studies of Metal Ions in Family II Pyrophosphatases: The Requirement for a Janus Ion, Igor P. Fabrichniy, ✗,§, Lari Lehtiö, ✗,#,§, Anu Salminen, ‖, Anton B. Zyryanov, ⊥, Alexander A. Baykov, ⊥, Reijo Lahti, ‖ and, and Adrian Goldman*, ✗ Biochemistry 2004 43 (45), 14403-14411 DOI: 10.1021/bi0484973.

A Novel Model to Identify Factors Associated with Mobility Apprehension

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Abstract **–** The prevalence of limb loss in the United States is estimated at 1.9 million instances with approximately 185,000 amputations occurring annually. Studies report that after lower-limb amputation, patients have high levels of pain, leading to restrictions in functional activity, and are at risk for developing avoidance behaviors. Potential contributing factors include muscle weakness leading to balance and gait abnormalities, which perpetuates low mobility and mobility apprehension. The aim of the study was to determine factors associated with mobility apprehension in individuals who underwent surgery for a lower-limb amputation. Adult participants who had a well-fitting prosthesis and had the ability to ambulate at least 10 feet were asked to participate in the study. Participants completed questionnaires regarding demographic information and self-report instruments measuring mobility apprehension. Mobility apprehension was measured using the Tampa Scale for Kinesiophobia (TSK). Of the participants, 65% reported a fall in the past year and 80% reported comorbidities (e.g. diabetes, high blood pressure, and heart disease). Linear regression analyses showed hip and core strength were independently related to balance ($r = .451$, $p = .046$; $r = .39$; $p = .085$; respectively). Fear of movement was not related to any of the study variables. Results may assist clinicians in aiming rehabilitation and exercise programs towards increasing hip and core strength, thus improving balance to promote better mobility for individuals with a lower-limb amputation. Further research is needed to identify factors that cause mobility apprehension.

I. Introduction

The prevalence of limb loss in the United States is estimated at 1.9 million instances with approximately 185,000 amputations occurring annually.[1] At this time, 54% of amputations are due to vascular dysfunction with a comorbidity of diabetes, 45% are due to traumatic injuries, and musculoskeletal tumors account for less than 2%.[2, 3] It is estimated that the number of people with an amputation will increase to 3.6 million by the year 2050,[3] about a 90% increase from current levels. If the incidence of limb loss due to vascular disease can be reduced by 10%, the number of people with amputation may be lowered by 225,000. Outcomes after limb loss appear to be variable. For instance, it is estimated that up to 55% of patients with an amputation due to diabetes will require an amputation of the second leg in two to three years.[1,3] An underwater treadmill walking program was developed and assessed to address this problem. This work has allowed insight into muscle weaknesses that limit mobility and increase fall risk. Establishing a connection between mobility apprehension and muscle weakness—and thus higher risk of falls—works in tandem with mitigating these factors via underwater treadmill training.

In an attempt to better understand the patient factors involved in post-amputation outcomes, cross-sectional and prospective cohort studies have investigated various demographic, clinical, physical, and psychosocial variables.[4-7] The most relevant patient factors include depression, comorbidity, fall risk, and poor quality of life.[8] Studies also report that after lower-limb amputation, patients have high levels of pain in the form of chronic low back pain, residual limb pain, and phantom pain leading to restrictions in functional activity.[6, 8]

Patients with high levels of pain[6] and disability[9] are at risk for developing avoidance behaviors. Vlaeyen et al.[10] developed the fear-avoidance model, which suggests that after an injury or surgery there are two pathways a patient will take based on his or her interpretation of acute pain. Pain as a result of injury or surgery that is perceived as non-threatening generally leads to the patient's recovery and return to normal activities of daily life. In contrast, pain that is perceived as threatening causes anxiety and induces a fear of movement. Fear of movement that continues beyond the expected healing time leads to avoidance behaviors. For amputees, fear of movement may take root during the lengthy recovery from surgery due to the pain and deconditioning that occurs. As a result, amputees are at risk for developing avoidance behaviors, which can then lead to greater pain, depression, and disability. Physical deconditioning may lead to a decrease in balance and an increased risk of falls, which subsequently increases avoidance behaviors and perpetuates the process.[10-14]

A theoretical model (**Figure 1**) similar to that of Vlaeyen et al.[10] is based on the factors related to high fall risk among lower-limb amputees.[15, 16] Potential contributing factors include muscle weakness that leads to balance and gait abnormalities, which subsequently perpetuates low mobility and a fear of falling.[15] Currently, fear of falling is measured by asking patients the question "Are you afraid of falling?". Although useful in many domains, this simple question is one-dimensional and does not provide robust data needed to develop exercise programs aimed at reducing the incidence of falls. The literature is lacking information pertaining to actual physical measurements that correlate psychological and physical function and discrepancies between the two. This study was designed to fill this knowledge gap by validating the Amputee Mobility Model (**Figure 1**) in order to identify factors involved in the deconditioning process and how these factors relate to specific patient attributes (reason for amputation, age, comorbidities, pain, etc.). With this information, a precision-medicine, customized exercise program can be developed based on the individual patient's needs, including cognitive behavior strategies to alleviate fears.

The overall purpose of this study was to determine factors associated with mobility apprehension in individuals who underwent surgery for a lower-limb amputation. Physical components included hip strength (left and right hip extensors), core strength (abdominal and back), and balance. The psychological component included fear of movement or mobility apprehension.[11] With knowledge gained from this proposed study, a customized healthcare (precision medicine model) exercise program can be developed. The aim of this study was to investigate the relationship between mobility apprehension, hip and core strength, and balance in people following lower-limb amputations. The hypotheses were as follows: there would be a negative correlation between self-reported mobility apprehension and balance and a positive correlation between hip and core strength and balance; mobility apprehension is inversely related to balance and strength.

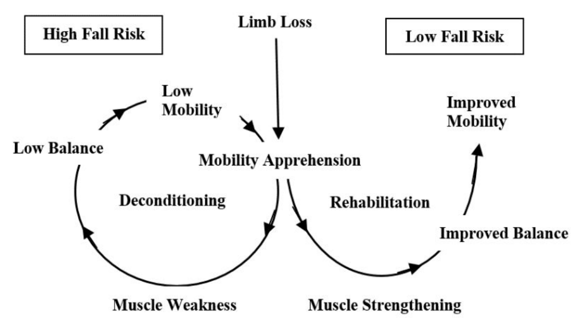

Figure 1. Amputee Mobility Model

II. Methodology

Subjects

A total of 20 English-speaking adults of both sexes and varied races with a lower-limb amputation were considered for study participation. Inclusion criteria were: 1) at least 18 years of age; 2) participants have a well-fitting prosthesis; and 3) have the ability to ambulate at least 10 feet. Exclusion criteria were: 1) advanced neurologic conditions severely affecting walking and balance; 2) musculoskeletal impairment of the contralateral limb that severely affects walking. Institutional Review Board approval was gained prior to data collection.

Experimental Procedure

Participant recruitment was performed at Fourroux Prosthetics in Huntsville, AL. Consenting participants completed an intake assessment to gather data on potential modifiers of response such as age, sex, race, ethnicity, education, and marital status. Other questions included date of surgery, reason for amputation, location of pain (no pain, stump, or phantom pain), duration of prosthesis use, history of falls, fear of falls, history of back pain, use of assistive devices, and comorbidities.[17]

Measurements

To determine mobility apprehension, participants were asked to complete the Tampa Scale for Kinesiophobia (TSK).[18-20] The TSK is a 17-item instrument that uses a four-point Likert scale, with scoring options ranging from 1= "strongly disagree" to 4= "strongly agree." A score of 37 is the cutoff score used to differentiate between those with high and low mobility apprehension. The TSK has been found to be a reliable index of fear of movement and has good internal consistency (Cronbach's alpha>0.70) and test-retest reliability (Pearson's r>0.70).

Hip and core strength were measured using a handheld dynamometer (JTech Commander PowerTrack II). Handheld dynamometry has been shown to be a reliable measure of impaired limbs of persons with disabilities.[22] The dynamometer was used to assess the strength of the trunk flexors, trunk extensors, and left and right hip extensors. All measurements were taken while the participant was sitting upright, supine, or prone on a treatment table. When performing the measurement, the participant was asked to perform one sub-maximal contraction into the dynamometer, and then told to relax[23]. The participant was instructed to hold onto the sides of the table to stabilize themselves when performing the contraction. Each measurement was taken three times, and the highest value was recorded. The testing position, movement direction, and placement of the dynamometer used in the current study is similar to the procedure of Thorborg, Peterson, Magnusson, and Holmich (2009).[23]

The Berg Balance Scale was the main outcome variable and has been shown to be a reliable measure of balance in persons with lower-limb amputation.[21] The 14 items consist of sitting to standing, standing with eyes closed, and turning 360 degrees.

Statistical Analysis

Mobility apprehension, hip strength, core strength, and balance were evaluated as means and standard deviations (SD). Separate linear regression analyses were performed to examine the relationships between balance and fear of movement and strength. Statistical significance was set at .10. IBM SPSS version 25 was utilized.

III. Results

Table 1. *Participant Characteristics*

Characteristic	**n (%)**
Mean Age (years, SD)	55.7 (10.1)
Sex	
Female	6.0 (30.0)
Male	14.0 (70.0)
Mean Years Since Surgery (SD)	9.6 (11.9)
Comorbidities	
≥1	16.0 (80.0)
None	4.0 (20.0)
Incidence of Falls in the Past Year	
Yes	13.0 (65.0)
No	7.0 (35.0)
Reason for Surgery	
Blood Clot	3.0 (15.0)
Infection	10.0 (50.0)
Trauma	2.0 (10.0)
Other	5.0 (25.0)

Table 2. *Scores for Mobility Apprehension, Balance, and Strength*

Characteristic	M (SD)
Mobility Apprehension (TSK)	38.65 (6.68)
High, ≥ 37 (n, %)	12.0 (60%)
Low, < 37 (n, %)	8.0 (40%)
Balance (BBS)	46.25 (6.74)
Hip strength in lbs. (SD)	15.78 (9.29)
Core Strength in lbs. (SD)	5.60 (3.17)

Table 3. *Correlation for Balance, Mobility Apprehension, and Strength*

Characteristic	Balance	TSK	Core	Hip
Balance	- -	0.10	0.39*	.45**
Mobility Apprehension (TSK)		- -	-1.9	-1.2
Core Strength			- -	.632**
Hip Strength				- -

Table 4. *Linear Regression for Factors Associated with Balance*

Characteristic	β (*p*)
Mobility Apprehension (TSK)	-.10 (.66)
Core Strength	.39 (.085)
Hip Strength	.451 (.046)

Of the participants (N=20), 65% reported a fall in the past year and 80% reported comorbidities (e.g. diabetes, high blood pressure, and heart disease). There were 15 participants with unilateral amputation (4 above the knee and 11 below the knee), and 5 participants with a bilateral amputation (all below the knee). The mean years since the date of the amputation was 9.6 (11.9) years. Participant characteristics are reported in **Table 1**. The mean Berg Balance score was 46.25 (6.74). The mean TSK score was 38.65 (6.68), where a score of ≥39 indicates a high level of mobility apprehension. The mean hip and core strength were 15.78 (9.29) lbs. and 5.60 (3.17) lbs., respectively. All study measurement data are presented in **Table 2** and correlations are presented in **Table 3**. There was a significant positive correlation between balance and hip strength and a positive correlation between balance and core strength. Linear regression analyses showed hip and core strength were independently related to balance ($r = .451$, $p = .046$; $r = .39$, $p = .085$; respectively). Fear of movement was not significantly related to any of the study variables.

IV. Discussion

The primary purpose of the study was to determine factors associated with mobility apprehension in individuals who underwent surgery for amputation of a lower-limb. In addition to quantifying the psychological factors associated with mobility apprehension, the current study was proposed to investigate the physical factors that may also be associated with mobility apprehension. Exposing and targeting factors associated with the mobility apprehension is imperative to reducing avoidance behaviors, improving strength and balance, and reducing the prevalence of falls.[10, 11] The results of this study are intended to help physical therapists, occupational therapists, and exercise specialists develop a customized rehabilitation or exercise plan to diminish those factors.

The hypotheses stated that there would be a negative correlation between self-reported mobility apprehension and balance and a positive correlation between core and hip strength and balance. It was also hypothesized that mobility apprehension would be inversely related to strength and balance. However, mobility apprehension was not significantly related to any of the study variables.

Analogous to the fear-avoidance model presented by Vlayen et al.,[10] we focused on exploring the deconditioning process after an individual undergoes an amputation, which precipitates decreased balance and low mobility. Our results showed significant findings correlating hip and core (abdominal and back) strength ($r = .45$; $p = .046$; $r = .39$, $p = .085$; respectively) to balance; therefore, the results support the model. This demonstrates the importance of postoperative strengthening in order to avoid muscle atrophy, specifically in the hip and core. There are currently no standardized strengthening exercise protocols for an individual who had just underwent surgery for a lower-limb amputation.

In concordance with previous studies,[24,25] it can be suggested that hip extensor strength is a predictor of gait patterns. Investigating multiple factors such as age, level of amputation, time since amputation, cause of amputation, strength, and balance, Raya, Gailey, Fiebert, & Roach,[25] found hip extensor strength to be the greatest predictor of distance walked in the six-minute walk test. Raya et al. also mentions the effect of hip extensor strength on balance and the balance insufficiency in the amputee population, resulting in a high prevalence of falls and fear of falling.[25] They postulated that individuals with stronger hip extensors and abductors have a more stable pelvis, resulting in the ability to control their prosthesis better. Therefore, individuals who are able to control their prosthesis show more symmetric gait patterns and are more efficient ambulators.[25]

In a 10-week strength training intervention, Nolan & Nolan[24] found that hip flexor and extensor strength were significantly increased in individuals with a lower-limb amputation. The study compared this group to another group of individuals with a lower-limb amputation who engaged in a 10-week period of occasional light activity, which resulted in a significant decrease in hip extensor strength. Thus, it can be deduced that it would be most beneficial for individuals with a lower-limb amputation to engage in a strengthening exercise intervention, in addition to occasional light physical activities, to gain and retain sufficient limb strength for improved balance and mobility and reduce the risk of falls.

Research also shows core strength to be an important indicator of balance. In order to balance out the forces produced by the upper and lower body, an individual needs sufficient core strength and stability.[26] Isometric hip external rotator and abductor strength can be used to determine core strength.[27] These muscles are correlated with a normalized prosthetic gait[28] and are often used in core stabilizing programs to improve the control of the lumbar-pelvic motion.[27]

Harvey et al.[28] posits that rehabilitation for individuals with an amputation should begin soon after the surgery, focusing on core muscles to increase body stability. An individual with insufficient core strength may be at risk for imbalances in their hips and lower extremities. This can result in joint damage and muscle weakness, which leads to physical and physiological alterations in the lower extremities. This can often perpetuate pain and result in an injury.[27] To reduce the risk of injury and potential falls, it is salient that individuals with a recent lower limb amputation engage in core-strengthening exercises after surgery.

Although not significant, the negative correlation between mobility apprehension and balance can insinuate a trend towards the validity of the Amputee Mobility Model. A study that implemented a 10-week dynamic balance training program for individuals ages 50-80, who were diagnosed with knee osteoarthritis showed improvements in self-reported fear of movement after undergoing the training program.[29] This study also reported high adherence rates—82.2%—which is important to consider when working with a population that may experience deconditioning and pain. Oksuz & Unal also found positive effects on self-report kinesiophobia (fear of movement), in addition to pain, functional status, and quality of life after prescribing Clinical Pilates exercises for six weeks to females with osteoporosis.[30] This is important when considering avoidance behaviors. Individuals engage in avoidance behaviors due to pain and the fear of re-injury. Rehabilitation and muscle conditioning of the limb leads to a reduction of fear, while deconditioning leads to muscle weakness, increased fear, and avoidance behaviors.[10] When these avoidance behaviors persist past the expected healing time, it may lead to physical and psychological detrimental consequences.[10]

Similar to the improvement of pain and functional disability following cognitive-behavioral therapy and physical therapy in populations with chronic low back pain, individuals with a lower limb amputation would benefit from protocols targeting mobility apprehension through a behavioral change model.[31] There is evidence that aerobic, balance, and strengthening exercises are beneficial for lower-limb amputees in improving mobility. Strengthening programs that implement cognitive behavioral therapy techniques aimed at reducing fear-avoidance tendencies reduces low back pain and improves compliance with rehabilitation.[11, 31] Aerobic training initiated early post-surgery benefits increased energy demand when walking with a prosthesis.[32] In addition to a previous study mentioned,[29] Mohamadtaghi, Hejazidinan, Shamsipourdehkordi, and Saemi demonstrated that performing specific balance exercises for one month does improve balance in individuals with a lower limb amputation.[33] Thus, a multicomponent exercise program consisting of aerobic, balance, and strength training coupled with a behavioral change model will enhance key functional weaknesses in this population.

Study Limitations

A few limitations should be considered when reviewing the current study. Because of the time frame and location of recruitment, we were limited in the number of subjects recruited. Although the direction of correlation was as hypothesized, with a nonsignificant p-value, the preliminary data explains the need for a larger sample size. Due to the small sample size, only one psychological variable was assessed in the study. In future studies, other variables such as pain catastrophizing (the tendency to ruminate and exaggerate pain situations), depression, and PTSD may need to be considered as an impactor on balance and mobility.[11] Although manual muscle testing has been validated for use in individuals with an amputation,[34] others have deemed it challenging to exert the correct amount of force to counter the muscle being measured.[35] However, to increase the reliability for the measurements, one rater performed all study measures.

In addition, although all subjects were evaluated postoperatively, the number of years since the subjects had the amputation varies. The least amount of time a subject had been out of surgery was four months, with the longest amount of time being roughly 49 years. Measuring individuals early after surgery will most likely provide a more accurate representation of the individuals' level of balance and mobility to determine the method for the strengthening program. Choosing participants with similar time since surgery will provide more conclusive results which will reduce the standard deviation for more robust statistical modeling. Future research is needed to determine the relationship between the number of years since surgery and the level of strength and balance.

V. Conclusion

The current preliminary study determines the importance and the applicability of the Amputee Mobility Model. It exposes the necessity of post-surgical implementation of strength and balance protocols to improve mobility in individuals who had undergone a lower-limb amputation. As seen in individuals with knee osteoarthritis and osteoporosis[29,30], evidence has shown that strength and balance programs have reduced mobility apprehension and risk of falls. Results may assist clinicians in aiming rehabilitation and exercise programs towards increasing hip and core strength, thus improving balance to promote better mobility for individuals with a lower-limb amputation. Further research is warranted to further identify factors that cause mobility apprehension.

VI. Acknowledgements

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References

1) Limb Loss Task Force/Amputee Coalition, Roadmap for Preventing Limb Loss in America: Recommendations from the 2012 Limb Loss Task Force. Knoxville, Tennessee; 2012.

2) Izumi Y, Satterfield K, Lee S, Harkless LB. Risk of reamputation in diabetic patients stratified by limb and level of amputation: a 10-year observation. Diabetes Care. 2006;29(3):566–70.

3) Ziegler-Graham K, MacKenzie EJ, Ephriam PL, Travison TG, Brookmeyer R. Estimating the prevalence of limb loss in the United States: 2005 to 2050. Archives of Physical Medicine and Rehabilitation. 2008; 89(3):422-9.

4) Sinha R, van den Heuvel WJ, Arokiasamy P, van Dijk JP. Influence of adjustments to amputation and artificial limb on quality of life in patients following lower limb amputation. International Journal of Rehabilitation Research. 2014;37(1):74–9.

5) Resnik L & Borgia M. Reliability of outcome measures for people with lower-limb amputations. Physical Therapy Journal. 2011;91(4):555-565.

6) Ehde DM, Czerniecki JM, Smith DG, Campbell KM, Edwards WT, Jenson MP, et al. Chronic phantom sensations, phantom pain, residual limb pain, and other regional pain after lower limb amputation. Archives of Physical Medicine Rehabilitation. 2000;81(8):1039–44.

7) Behel JM, Rybarczyk B, Elliott TR, Nicholas JJ, Nyenhuis D. The role of perceived vulnerability in adjustment to lower extremity amputation: a preliminary investigation. Rehabilitation Psychology. 2002;47(1):92–105.

8) Sinha R, van den Heuvel WJ, Arokiasamy P, van Dijk JP. Influence of adjustments to amputation and artificial limb on quality of life in patients following lower limb amputation. International Journal of Rehabilitation Research. 2014;37(1):74–9.

9) Penn-Barwell JG. Outcomes in lower limb amputation following trauma: A systematic review and meta-analysis. Injury. 2011;42:1474-1479.

10) Vlaeyen J, Kole-Snijders A, Boeren R, van Eek H. Fear of movement/(re)injury in chronic low back pain and its relation to behavioral performance. Pain. 1995;62:363-372.

11) Archer KR, Seebach CL, Mathis SL, Riley LH, Wegener ST. Early postoperative fear of movement predicts pain, disability, and physical health six months after spinal surgery for degenerative conditions. The Spine Journal. 2014;14:759-767.

12) Archer K, Mathis SL, Vanston S, Devin C, Spengler D, McGirt M, Cheng J, Aaronson O, Wegener S. A cognitive-behavioral based self-management approach to physical therapy: improving surgical spine outcomes. The Journal of Pain. 2014;15(4):S110.

13) Archer KR, Devin CJ, Vanston SW, Koyama T, Phillips SE, George SZ, Mathis SL McGirt MJ, Spengler DM, Aaronson OS, Cheng JS, Wegener ST. A cognitive-behavioral based self-management approach to physical therapy: improving surgical spine outcomes. The Journal of Pain. 2016;17(1):76-89.

14) Yu JC, Lam K, Nettel-Aguirre A, Donald M, Dukelow S. Incidence and risk factors of falling in the postoperative lower limb amputee while on the surgical ward. Physical Medicine and Rehabilitation. 2010;2(10):926-34.

15) Miller WC, Speechley M, Deathe B. The prevalence and risk factors of falling and fear of falling among lower extremity amputees. Archives of Physical Medicine and Rehabilitation. 2001;82(8):1031-7.

16) Kulkarni J, Wright S, Toole C, Morris J, Hirons R. Falls in patients with lower limb amputations: Prevalence and contributing factors. Physiotherapy. 1996;92(2):130-36.

17) Melchiore PJ, Findley T, Boda W. Functional outcome and comorbidity indexes in the rehabilitation of the traumatic versus the vascular unilateral lower limb amputee. American Journal of Physical Medicine and Rehabilitation. 1996;75(1):9-14.

18) French DJ, France CR, Vigneau F, French JA, Evans RT. Fear of movement/(re)injury in chronic pain: A psychometric assessment of the original English version of the Tampa Scale for Kinesiophobia. Pain. 2007;127:42-51.

19) Roelofs J, Goubert L, Peters M, Vlaeyen J, Crombez G. The Tampa Scale for kinesiophobia: further examination of psychometric properties in patients with chronic low back pain and fibromyalgia. Eur J Pain. 2004;8:495-502.

20) Swinkels-Meewisse E, Roelofs J, Schouten E, Verbeek A, Oostendorp R, Vlaeyen J. Fear of movement/(re)injury predicting chronic disabling low back pain: a prospective inception cohort study. Spine. 2006;31:658-664.

21) Major MJ, Fatone S, Roth EJ. Validity and reliability of the Berg Balance Scale for community-dwelling persons with lower-limb amputation. Archives of Physical Medicine and Rehabilitation. 2013;94(11):2194-2202.

22) Riddle D, Finucaine S, Rothstein J, Walker M. Intrasession and intersession reliability of hand-held dynamometer measurements taken on brain damaged patients. Physical Therapy. 1998;69(3):182-94.

23) Thorborg, K., Petersen, J., Magnusson, S., & Holmich, P. (2010). Clinical assessment of hip strength using a hand-held dynamometer is reliable.(Report). Scandinavian Journal of Medicine and Science in Sports, 20(3), 493–501. https://doi.org/10.1111/j.1600-0838.2009.00958.x

24) Nolan, L., & Nolan, L. (2012). A training programme to improve hip strength in persons with lower limb amputation. Journal of Rehabilitation Medicine, 44(3), 241–248. https://doi.org/10.2340/16501977-0921

25) Raya, M. A., Gailey, R. S., Fiebert, I. M., & Roach, K. E. (2010). Impairment variables predicting activity limitation in individuals with lower limb amputation. Prosthetics and orthotics international, 34(1), 73-84.

26) Martens, J., Einarsson, I. P., Schnizer, N., Staes, F., & Daly, D. (2011, June). Lower trunk muscle activity during front crawl swimming in a single leg amputee. Portuguese Journal of Sports Sciences (Vol. 11, No. Suppl. 2, pp. 751-754). Universidade do Porto, Faculdade de Ciencias do Desporto e de Educacao Fisica.

27) Sarvin Salar, Hassan Daneshmandi, Mohammad Karimizadeh Ardakani, & Hossein Nazari Sharif. (2014). The Relationship of Core Strength with Static and Dynamic Balance in Children with Autism. Annals of Applied Sport Science, 2(4), 33–42. https://doi.org/10.18869/acadpub.aassjournal.2.4.33

28) Harvey, Z. T., Loomis, G. A., Mitsch, S., Murphy, I. C., Griffin, S. C., Potter, B. K., & Pasquina, P. (2012). Advanced rehabilitation techniques for the multi-limb amputee. Journal of surgical orthopaedic advances, 21(1), 50.

29) Takacs, J., Krowchuk, N., Garland, S., Carpenter, M., & Hunt, M. (2017). Dynamic Balance Training Improves Physical Function in Individuals With Knee Osteoarthritis: A Pilot Randomized Controlled Trial. Archives of Physical Medicine and Rehabilitation, 98(8), 1586–1593. https://doi.org/10.1016/j.apmr.2017.01.029

30) Oksuz, S., & Unal, E. (2017). The effect of the clinical pilates exercises on kinesiophobia and other symptoms related to osteoporosis: Randomised controlled trial. Complementary Therapies in Clinical Practice, 26, 68–72. https://doi.org/10.1016/j.ctcp.2016.12.001

31) Van Tulder, M. W., Ostelo, R., Vlaeyen, J. W., Linton, S. J., Morley, S. J., & Assendelft, W. J. (2000). Behavioral treatment for chronic low back pain: a systematic review within the framework of the Cochrane Back Review Group. Spine, 25(20), 2688-2699.

32) Esquenazi, A., & DiGiacomo, R. (2001). Rehabilitation after amputation. Journal of the American Podiatric Medical Association, 91(1), 13-22.

33) Mohamadtaghi, M., Hejazidinan, P., Shamsipourdehkordi, P., & Saemi, E. (2018). The effect of a balance training program with and without mirror on postural control of lower limb amputees. International Journal of Applied Exercise Physiology, 7(4), 50-61.

34) Leijendekkers, R. A., Gerben, v. H., Sman, A. D., J, B. S., Maria W G Nijhuis-van der,Sanden, & Hoogeboom, T. J. (2017). Clinimetric properties of hip abduction strength measurements obtained using a handheld dynamometer in individuals with a lower extremity amputation. PLoS One, 12(6) doi:http://dx.doi.org.elib.uah.edu/10.1371/journal.pone.017988735)

35) Lunsford, B. R., & Perry, J. (1995). The standing heel-rise test for ankle plantar flexion: criterion for normal. Physical Therapy, 75(8), 694-698

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