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The Journal of Undergraduate Research (jur) is dedicated to providing the student body with intellectual perspectives from various academic disciplines. jur serves as a forum for the presentation of original research thereby encouraging the pursuit of significant scholarly endeavors.

From the Editors

Dear Rochester Community,

The University of Rochester offers numerous opportunities beginning with entering freshmen and extending throughout a student's undergraduate years. Any individual with the passion, drive, and curiosity to perform research and expand his or her interests can be involved in research; University of Rochester faculty members encourage and cultivate an environment that makes this a possibility.

The Journal of Undergraduate Research maintains its focus on exemplary work at the College, though new students and new research surface each semester. We strive to present the finest, noteworthy undergraduate research in both quality and interest to the academic community. Our constancy provides the basis for creativity and innovation and allows for progression. Our university motto is melliora—"ever better"—and we aim to embody this ambition and flexibility.

The Journal of Undergraduate Research has worked extensively to incorporate new endeavors and an accessible layout. We have included research from a variety of disciplines that displays the University of Rochester's multifaceted personality. Our journal aims to highlight students' research achievements from across the College of Arts and Sciences and the Hajim School of Engineering and Applied Sciences. We continue to work with our student organizations and supporting departments across the University of Rochester to produce our best possible journal each season.

Sincerely,

Jessica Gambacurta and Elizabeth Tien
Editors-in-Chief

Jur

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Perspectives on Research

Richard M. Ryan, Ph.D.

Professor of Clinical and Social Sciences in Psychology

JUR: How did you get started in this profession?

As an undergraduate, I started out as a math major. I did well in math and enjoyed math, but I had no idea what to do with math. As a child of the early 70s, questions of meaning and what the purpose of all of this was were looming large for me. When I did not know what I was doing with my studies, I dropped out of school, sort of to figure out the meaning of things on my mind. Shortly thereafter, my father discovered me. This turned out to be a very good thing, because I really was out on my own to ask those questions; I did not have to worry about what somebody else was thinking – that had already been taken care of.

I moved to Cambridge with my girlfriend at the time, and that was a really intellectually rich environment at the time, even though I was just working as a factory wrecker. There were bookstores everywhere; you could find a philosopher or go to a talk on any corner. As a result, I got very interested in philosophy, and I started reading a lot. For the first time in my life, instead of just wanting to do well in school, I was actually interested in what I was reading and I wanted to know something. So that is when I had my first inklings of what it means to have passion for intellectual matters.

After a year of working, I decided I wanted to go back to school and study philosophy, because that dealt with the burning questions, the existential questions – questions about history and culture and shaping ourselves. So I went back to school hungry for knowledge. Through philosophy, I was introduced to epistemology, which got me interested in psychology and how people learn and know what they know. Most of my courses in psychology were about learning, learning theory, and clinical matters, because I was interested in Freud, organismic theories and dialectics.

JUR: How did you get involved in research?

I think what was really cool about all of that was that I really became an intellectual at the time, and that's what I find most interesting about psychology – that it allows you to ask really deep questions and be interested in the things that really matter in life, and make a living at the same time. That's kind of a striking thing, because for most people they have to divide those two things up – life is not work and work is not life. But for me, those two things were a melded passion at the time.

I had no intention of becoming a researcher, but I lucked into becoming a psychologist. I was unemployed after graduating; you know, what does a philosophy major do after college? They go jobless. You can open up a paper, and there are no positions for wages available. I was living with my partner at the time, and she told me I needed to get a job. She told me there were aide positions available at the local institution where she was working. I didn't know what an aide did, so I put on a tie and jacket, typed up a resume, and went to apply for the job. Because I had gotten dressed up, they thought I was there for a job as a director of a pretty big program that they had there. In that interview, I ended up getting the job, even though I wasn't supposed to be there. That was nice on several levels, to be able to come home that night and tell Myriam that I did get a job, and I think I'm your boss. That's always been a good one between us.

When I began that job, they gave me a budget to hire some masters-level psychologists. We worked with developmentally delayed clients, and the only thing they understood about how to motivate our clients was to use behavioral techniques. But those never seemed to work – they never seemed to last. They would only work when you were standing around with the reinforcements – they hardly ever generalized. I just didn't like the methods, and I thought, jeez, can't they think more deeply about this? There are other ways of hooking people's energies and initiatives, even these people who are delayed – after all, they also have intrinsic satisfaction.

So without even having any of those words in my head, I knew this was not the way to go about things. And we set up a program that was not built around that, and the two psychologists got it too. My whole idea was that natural reinforcements and natural, normalized environments were motivating in and of themselves; that people like to have capabilities, and that is in itself a reward.

That gave me the idea that psychology seems pretty simplistic. As underhanded as it seems, I thought even I could do that. So I applied to graduate schools in psychology after having this experience and watching psychology in action, but only with the intention of becoming a therapist. I was very into reading humanistic and existential and psychodynamic writings. But I had a rude awakening when I came [to the University of Rochester] for graduate school,

because it was all behaviorism there. So I cried everyday to my now wife at the time, asking why I had decided to go here. I was being trained in things I didn't agree with, like, or think was very good, but I also knew I had to do it. I guess from a psychodynamic point of view, I was also interested in why I felt so resistant to those things anyway.

So I decided to learn everything, even the things that were unpalatable to me, as part of my training. I was doing research on brain potentials, more or less just to have some research to do, because I was required to as a clinical student. But while I was here, after having gone through a small compliant period, I decided I have some really deep questions to ask, and I want to get at them.

Ed Deci was here at the time; he and I used to have some pretty deep conversations about things. We collaborated on a paper or two that made it, somehow; so I had the opportunity to become an academic. I wasn't sure I wanted to do it, but I decided I was having fun doing research and I could get a job as an academic. There's this sort of thing where, if you go academic and you change your mind, you can always go be a clinician. But if you become a clinician, and you change your mind, you can't really go be an academic. So I decided I was going to give this a shot and see how it goes, and when it stops being fun, I'll go open up a private practice.

JUR: Do you do any clinical work right now?

Yes, I have a part-time practice. I only see a few people per week, but that's mostly to keep my hand in, because I'm the principal supervising boss of the psychotherapists, so I need to keep my hand in. It's kind of my art and craft, and it has to be honed.

JUR: Why, then, is research important to you?

Part, it's fun. It's an interesting thing to do. You get to ask questions and work with students, and do that kind of thing. But more seriously than that, something that always struck me about being a private practice clinician is that you can only reach those individuals that you see. And your work can only have the impact that it radiates from the impact that you have on those people. And as somebody who is really interested in social change and making a difference in the world, I'm not sure that was enough for me. The work we do on Self-Determination Theory, however, reaches thousands of peoples, and has informed practices, businesses, schools and clinics, or at least it has the potential to do so. So it really has to do with the magnitude of impact you can have as a scholar, versus the magnitude of impact you can have as a clinician. Being a clinician is very intense and personal, but you deal with very few people.

There's a guy, Edward Albee, who was a community psychologist back in my day. Community psychology has died as a field, but part of the birth of community psychology was out of Albee's analysis, which is that even if therapists were completely successful with everybody they saw, they could not have an impact on the mental health of the country. There just aren't enough clinicians to go around, and that's no way to make social change happen. So I think part of it is just that I've always been interested in dialectics in society, and seeing the issues there. It's really a question of where you have leverage.

JUR: What fields and applications are you currently looking into?

Because Self-Determination Theory (SDT) is a general theory of motivation, well-being and development, it has applications all over the place. I have burning interests in several areas, and at any given time, I have 15-25 projects up and running. They're pretty diverse in terms of their focus. One of my graduate students, Netta, is doing work in mindfulness, stress, prosocial behavior and nature. Another graduate student of mine, Andy, is doing work with video games and virtual environments. I have work going with James Maiciale on religious motivation. I am working with my collaborators in Israel on parental conditional regard, and parenting processes in socialization. I have work going with a former student of mine, Valery Chirkov on cross-cultural applications of SDT. I have work going with our former post-doc Seungmi Yang on educational processes in Korea and the similarity of our models with what's going on there. We've also done work on vitality, sports, smoking cessation, and a virtual clinician. This is an incomplete list, but in all of these domains I probably have a project going. My sort of role in Self-Determination Theory is to keep a lot of things going; to be an idea person and grease the wheels in the various places, and be a finisher more than a doer, because I can't be doing it all.

JUR: From your perspective, how has your field progressed?

When I got involved in psychology, motivation wasn't even a field. In fact, it had been declared by a number of behaviorists and psychologists to be a dead former field of psychology. But I never felt that way about it. Motivation has come back into the heyday now. I'm not going to take a lot of credit for it, but Self-Determination Theory has had some role in that, along with a couple of other theories. So motivation has come back, and I would say it's a big question for people. And as motivation has moved from a peripheral topic to a mainstream topic, our work has gone from a peripheral theory to a mainstream theory. And that was recognized by people; even if they don't like it, it's something to contend with. So in the sense of visibility, interest and receptivity, the field has moved a lot.

The field has also advanced dramatically in terms of the perplexity of the questions being asked and the tools being brought in to answer those questions. Some of that is due to changes in statistics. We've gone from regression being the most advanced technique that was out there, to structural equation modeling, to multilevel hierarchical modeling, so that the things you can even ask about are different. The tools for implicit and explicit motivation have been developed in the meantime, because there was no way of doing those things before. So it's really that methods develop, and methods allow you to ask more sophisticated questions. They go hand in hand, and this is why I like being a quantitative researcher, rather than a qualitative researcher. In quantitative research, the methods can often refine the way you think. The methods can come back and teach people new things. And I'm getting taught new things all the time.

JUR: What are the most significant roadblocks you've encountered in research?

I think the main one is grant money. There's always the necessity of funding.

JUR: What about personal obstacles?

Self-discipline. It takes persistence. One difference about being a clinician and a researcher is that the satisfactions of clinical work are immediate, they are right in front of you all of the time. The

satisfactions of doing research are always delayed. The magnitude is bigger, but the delay is longer, so in terms of reinforcement value, you have to have a lot of willingness to delay gratification to be a researcher. You've got a lot of work to do before you get anything at the end of it, and a lot of organizational tasks. It's better for me now, because I don't do a lot of that stuff, but I had to begin there. And even now, it's a different kind of mundane thing, so I have just as many mundane things to do. And I have to have the discipline to do those things, even if they are not growth-promoting for me. So for me, finding the discipline to do the mundane, because I'm somebody who really loves the ideas, and somehow doing the mundane stuff, that's much harder for me.

JUR: What advice do you have for students who find themselves in a similar situation as you, asking deep questions and searching for those answers?

One thing I think I'd first like to say is that I don't think that ends. Once you've already opened up that existential capability, hopefully that doesn't end. I suppose on one level people think, 'well, I'll find the answers and things will be settled,' but I think it's more that you keep refining your question. I feel really lucky in life, that I had never planned a career. I was pursuing the things that interested me, and a career happened. I think that's the best of all worlds.

That's one nice thing about psychology, this discipline is about us. People we know in our everyday experience. That's really a privilege to be occupied with such things.

Unassuming Heroism[1]

Richard Allen's Theology, Writing and Leadership during Philadelphia's Yellow Fever Epidemic of 1793

David Liebers, 2009
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1. This phrase was taken from Celebration by William Rickell commenting a letter written by Dr. Benjamin Rush who wrote of Richard Allen's actions during the Yellow Fever Epidemic of 1793 in Philadelphia. William Renwick Riddell "An Instance of Humanity of the Negro" *The Journal of Negro History*, 2, (Apr. 1929), 238.

Historical moments of catastrophe have a dual nature. Instances of great tragedy and transcendent obstacles afford the possibility that racial barriers might be jettisoned. Philadelphia's Yellow Fever epidemic of 1793 was such a case. Highlighting the destruction and injustice of the event, Absalom Jones and Richard Allen, the religious and social leaders of black Philadelphia, invoked this proverb in a refutation of censures directed at the black community: "When war is over, and all things righted, God is forgotten, and the soldier slighted."[2] The anxieties, acts of heroism, written words, and dialogue generated by the event provide a case study in early Black historiography and social leadership. Although the service of blacks from Philadelphia and their efforts were largely marginalized, the epidemic prompted Philadelphia's nascent free black population to develop an internal community dynamic, fostering the growth of black institutions. Alongside these institutional developments during this transitional period between slavery and freedom, Richard Allen's shrewd, prophetic voice gave birth to a generation of activism and protest.

In August of 1793, Dr. Benjamin Rush, signer of the Declaration of Independence and one of America's eminent physicians, wrestled with a mysterious illness plaguing several Philadelphians. Symptoms included fever, a morbid yellow coloring of the skin, and black granular vomit. In accordance with the prevailing miasmatic theory of disease transmission, Rush's initial reaction was to blame the "noxious effluvia of rotting coffee," and the "pestilential wharf" that seemed to connect the cases. His explanation started to deteriorate as people in other parts of the city succumbed to the same disease. With the memory of an epidemic thirty years prior on the minds of Philadelphians, few proclamations could have pained the city more: "the bilious remitting yellow fever"[3] was making another appearance. In the immediate wake of his announcement, many dismissed Rush's worries as alarmist, but the coming months would confirm his diagnosis.[4] Within a few weeks, havoc wreaked the city and those with the means fled. Rush began to pour through all prior records of the fever. He found evidence that blacks were immune to the yellow fever from an account of a previous epidemic in South Carolina, and suggested to Richard Allen and Absalom Jones that they would offer their services in procuring nurses to the sick and burying the dead.[5] "Heeding a Christian commitment to mutual aid," and feeling a duty to the doctor who had long been their ally, they responded to Rush's call.[6] On September 9th, in a notice in the *Daulap's American Daily Advertiser*, Allen announced the availability of the services of black people, "under a greatful remembrance of the favours received from the white inhabitants."[7]

The language of disease immunities and susceptibilities has long been associated with the issue of race, and both used to justify slavery. The evolution of African slavery is inextricably linked to the evolution of race as a historical construct. Before the establishment of plantation race-based slavery, Africans, Atlantic crooks, European indentured servants, and Amerindians comprised an ethnically heterogeneous enslaved community in the Americas. Because of previously acquired disease immunities from the African tropics, "blacks were nominated for labor in the tropics quite literally by the process of elimination, and the notion that only Africans could perform labor in hot climates was born."[7] These arguments were recycled in the late antebellum period by Southerner slaveholders defending their belief in the innateness of their peculiar institution of slavery. On the other hand, disease susceptibilities led to a medi-

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1. Richard Allen. *The Life, Experience, and Gospel Labours of the Rt. Rev. Richard Allen.* (Philadelphia: Martin & Boden, Printers, 1833), 42. (Transcribed by UNC Chapel Hill) and available on-line with support from the National Endowment for the Humanities. Included in this publication was the *Narrative of the Yellow Fever epidemic* co-written with Absalom Jones.

2. John H. Powell, *Bring Out Your Dead*, (New York: Time Life Books, 1965), 13.

3. Ibid., 12.

4. It is possible that those people of color who were born in the Caribbean or West Africa might have increased resistance to Yellow Fever, especially if they were exposed to the disease previously. However, the final figures of those deceased are not particularly persuasive to this possibility in the case of Philadelphia in 1793. See page 38 of Allen's *Narrative*.

5. Phillip Lapansky, "'Abigail, A Negress': The Role and the Legacy of African Americans in the Yellow Fever Epidemic," in *A Melancholy Loss of Dissolution*. J. Worth Estes and Billy G. Smith. (Philadelphia: Science History Publications, 1997), 63.

6. *Daulap's American Daily Advertiser* September 9, 1793 (Philadelphia).

7. Kenneth Kiple and Virginia Kiple, "The African Connection: Slavery, Disease and Racism," *Phylon* 41 (1980): 213.

cal lexicon that categorized "black diseases," and overall, elevated "prejudice to the level of science, thereby giving it respectability." [6]

Richard Allen, a former slave, was an important clergymen and organizational leader for Philadelphia's black population. Description of Allen's youth is scant. He was born in Philadelphia in 1760, and as a consequence of several transactions was separated from his mother at a young age. [7] After securing manumission, he took his name and worked his way through a half-dozen states on the Methodist preaching circuit. [8] Returning to the city of his birth, Allen set out to fulfill his goal of building an Afro-Christian constituency and working to mitigate the system of bondage into which he was born. Though many whites distrusted those blacks who were taking care of the sick and the dead at the risk of their own lives, Rush, at least in his writing, says he was convinced of racial equality in both moral and intellectual spheres. [9] Rush wrote extensively on the merits and healing skills of his friend Dr. James Durham, a former slave, and the skill of his own servant, Marcus, as equaling "any apothecary in town." [10] The intentions of Rush's call for aid are not clear, but the response fitted into a longstanding pattern of black people being forced to bury the dead and other noxious forms of employment. The Union Army's use of black soldiers for this purpose on the battlefields of the American Civil War is perhaps the most widely known example. For this reason, it is difficult to appraise Rush's motives in asking Allen for aid. My concern is less with whether or not Rush's act was "racist" and more with the response of the black community.

Portents of change reached Philadelphia in th elate 18th century. In 1780, the bill for gradual abolition passed. By 1800 fewer than one-percent of the black population was enslaved. [11] The same year that the United States Constitution was written and adopted in Philadelphia, a group of former slaves, "considering their place in the new republic and their relationship to Africa," organized into what was essentially an early conception of the Freedman's Bureau in the form of the Free African Society, a social organization whose conception was partly orchestrated by Richard Allen. [12] In the late summer of 1793, black men and women entered a unique urban landscape. Not only were the majority of them free, black institutions were beginning to take foothold in the city. For a brief moment, white leadership presence was absent or suppressed by a force beyond its control. It was in this stage that black leaders tested their organizations. Common black men and women experienced a new environment, even while performing tasks to which they may become accustomed to. The temporal proximity of this event to the institution of slavery in Philadelphia, which was widespread just two decades earlier, is significant. [13] Though the noxious work performed by the black community was work they may have performed as slaves, they now did it as freed men and women in the context of free black institutions and leadership. Freedom, however, was not the ultimate prize for all blacks. Blacks, free and enslaved, operated in a society that severely limited their social and physical mobility. Legislation and prevailing attitudes did not automatically change with the passage of the gradual abolition bill in 1780. For example, The Fugitive Slave Act of 1793 infringed freedom's meaning for blacks—even the well known Allen was accused of being a runaway in 1806. [14, 15]

The Yellow fever wrought havoc in the summer and fall months of 1793—having killed thousands, displaced the federal government, and stalled public life. In the aftermath, Mathew Carey, an Irish-born businessman and economist, wrote *A Short Account of the Malignant Fever*, his reflections on the epidemic. Carey detailed modes of treatment, the flight of Philadelphia's citizens, and the general state of despondency. He also made room to chide the black community. "They extorted two, three, four, and even five dollars a night for such attendance, as would have been well paid for, by a single dollar," explained Carey in reference to poor blacks and whites, "some of them were even detected in plundering the houses of the sick." [16] Carey refers here to both poor whites and blacks, but the implication is clear. He characterizes the response of black people as exploitative and greed-driven. Carey's *Short Account* was wildly popular. The first edition sold out within days, and "in all, he published and sold over 10,000 copies." [17] The idea of poor black people throwing aside prior injustices and responding to Dr. Rush's call for help did not fit the narrative that Carey hoped to craft. Instead, he highlighted out a public meeting of the few city officials who did not flee. In Carey's account, the meeting hosted by Mayor Matthew Clarkson was an iconic moment that created order from chaos, referring "back to previous constitutive moments in American civic mythology; among them the Mayflower Compact, the Declaration of Independence, and the Constitutional Convention." [18] It is hard to imagine Richard Allen, described as a "shrewd, quick, popular leader, positive and dogged, and yet facetious in his knowledge of Negro character," acquiescing to such a description. [21] His response soon followed. [22]

The *Narrative of the Proceedings of Black People, During the Late Awful Calamity in Philadelphia*, written by Richard Allen and Absalom Jones in response to Mathew Carey's derisive comments provides us with

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8. Ibid., 216.

9. Richard S. Newman, Freedom's Prophet: Bishop Richard Allen, the AME Church, and the Black Founding Fathers. (New York: New York University Press, 2008), 78.

10. Ibid., 45.

11. Donald J. D'elia, "Dr. Benjamin Rush and the Negro," *Journal of the History of Ideas*, 30 (1969): 414

12. Ibid., 415.

13. Susan E. Klepp, "Seasoning and Society: Racial Differences in Mortality in Eighteenth-Century Philadelphia," *The William and Mary Quarterly*, 3rd Ser., Vol.51 (Jul 1994): 495.

14. Gary B. Nash, Forging Freedom : *The Formation of Philadelphia's Black Community*, 1720-1840. Harvard University Press, 1988, 100.

15. Gary B. Nash, "Slaves and Slaveowners in Colonial Philadelphia," *The William and Mary Quarterly*, 3 (1973).

16. An Act respecting fugitives from justice, and persons escaping from the service of their masters (1793), as enacted by the Senate. Made available on-line at: (http://academic.udayton.edu/race/02rights/slave02.htm)

17. Gary Nash has cited and discussed the kidnapping confrontation of Richard Allen in 1806, although its legitimacy is somewhat dubious and is perhaps apocryphal. Nash, *Forging Freedom*, 247.

18. Carey, *A Short Account*.

19. Sally F. Griffith, "A Total Dissolution of the Bonds of Society: Community Death and Regeneration in Mathew Carey's Short Account of the Malignant Fever" in *A Melancholy Scene of Devastation*, J. Worth Estes and Billy G. Smith. (Philadelphia: Science History Publications, 1997), 47-48.

20. Ibid., 51.

21. W.E.B. DuBois, *The Philadelphia Negro* (Philadelphia: University of Pennsylvania Press, 1996), 21.

22. Absalom Jones and Richard Allen, *A Narrative of the Proceedings of the Black People, during the Late Awful Calamity in Philadelphia, in the Year 1793: And a Refutation of some Censures, Thrown upon Them in Some Late Publications* 1794

an instance of a profound and novel articulation, demonstrating how black leaders defended a people whose stories deserved to be told. This was the first document of African-American authorship to be granted federal copyright. [23] While distinctions of primacy are often dubious as they can be qualified in so many ways, this particular distinction speaks to two issues. First, it represented successful petitioning to, and co-opting of, governing bodies to project a call for justice. Second, it helped to push the role of the black leaders and abolitionists into the print media, where it would grow in popularity (with varying success) in the coming decades.

Allen and Jones continuously advertised their publication in the Philadelphia's *General Advertiser*. Interestingly, advertisements for Mathew Carey's various writings (including his popular account of the Yellow Fever epidemic) are scattered around Allen and Jones' advertisements on a consistent basis, as if in hopes to minimize its impact. [24] Although Allen and Jones relied heavily on interaction with their white neighbors, in this influential response, these two men claimed agency for black people in the healing of a great many Philadelphia citizens. "We have been the instruments, in the hands of God, for saving the lives of some hundreds of our suffering fellow mortals," [25] aware of the moral clarity of their purpose and sure of their efforts. True to his nature, Allen's tone is courteous, yet unwavering. He saw an injustice and acted upon it with diligence, eloquence, and a sense of purity. In the *Narrative*, Allen accomplished a number of monumental feats. Aside from claiming the first black federal copyright, it addressed serious issues of black historiography and leadership. Its publication included notes on the institution of slavery. Noting in particular that "it is foolish that a superior good character is looked for, from our race, by those who stigmatize us as men, whose baseness is incurable, and may therefore be held in a state of servitude, yet you try what you can to prevent us from rising from the state of barbarism you represent us to be in." [26] This contradiction remains a central theme in the study of the dynamics of race and slavery and is representative of the frustration felt by black leadership in the instance of the 1793 epidemic.

Biographical difficulties arise in discerning any substantiative character flaws; Allen might have had from his or his contemporaries' writing. He was widely embraced and looked up to as a moral compass for the African-American community. If anything, he was too overbearing in his yearning for justice in the eyes of God,and disenchanted more than a few of his acquaintances with his "unyielding, stern, and overbearing," tendencies. [27] One can imagine Allen confronted ill-doers in the street, giving them impromptu sermons awash in biblical allusion and fervor, and then sending them on their way.

Cognizant of the challenges faced by a black leader in a racist society, it is not altogether surprising that Allen stressed importance of black political and economic institutions. Gary Nash has argued that Allen's years of traveling and preaching between 1780 and 1786 "[seem] to have increased enormously his confidence at maneuvering in a world dominated by whites." [28] The Yellow fever epidemic of 1793 was at the apex of another journey; Allen had led the charge in the development of a black Philadelphia and black Atlantic world. W.E.B. DuBois addressed the actions of these visionary leaders in *The Philadelphia Negro*, specifically highlighting Allen and Jones:

> "These two were real leaders and actually succeeded to a remarkable degree in organizing the freedmen for group action. Both had bought their own freedom and that of their families by hiring their time—Allen being a blacksmith by trade, and Jones also having a trade. When, in 1793, the terrible epidemic drove Philadelphians away so quickly that many did not remain to bury the dead, Jones and Allen quietly took the work in hand, spending, some of their own funds and doing so well that they were publicly commended by Mayor Clarkson in 1794." [29]

Though the epidemic was not the first instance of Allen and Jones engaging in humanitarian efforts— indeed they had just finished work on the African Episcopal Church of St. Thomas which was dedicated just as the disease began to spread—the events of 1793 tested them in their roles and gave them an opportunity to articulate the definition and model of black leadership.

W.E.B. DuBois' comments on the work of Allen and Jones suggested that there has been an alternative black historiography of the events of the 1793 epidemic, but it certainly finds its roots in the *Narrative*. However, "it is misleading to suggest simply that African American historians [were] faced with the task of correcting an inaccurate historical record—for there was no singular record, and the inaccuracies in the record were manifestations of a deeper problem." [30] Allen, instead, was a player on an international stage that featured a discussion on the nature of leadership and the moral imperative of abolition. Furthermore, Allen inaugurated a tradition of African-American publication and historiographical agency. The successes and achievements of the Black clergy and leadership should not overshadow the legacy of those who they represented. Philadelphia was influenced by its Quaker roots, and its Abolition Society was a model for all other such societies in the country. In general, it was borne to "great champions of the people of color," including Reverend James Sproat and Dr. Benjamin Rush. These factors, among many others, made Philadelphia's black population one of the most educated and literate in the United States. The usage here of the terms "black population" and "black community," in reference to Allen's constituency, simplified as a complex group of people and should be clarified. From slaves to intellectuals, Philadelphia's blacks occupied a spectrum of societal niches. However, most freedmen had few economic opportunities and no social equality. Women were domestic workers and men took jobs as day laborers or tradesmen. [31] The "black community" was comprised of a variety of African, Caribbean, and American tradition, each adding "their distinctive contents, their accents, and their perspectives to the diversity of the city's black population." [32]

Richard Allen was careful about accepting personal praise, and consistent in giving it to the common black men and women whose

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23. Newman, 78.
24. *General Advertiser*, February 8,, 1794.
25. Allen, 29
26. Ibid., 45.
27. Ibid., 176
28. Gary B. Nash,"New Light on Richard Allen: The Early Years of Freedom," *The William and Mary Quarterly*, 46 (1989), 338.
29. W.E.B. DuBois, *The Philadelphia Negro* (Philadelphia: University of Pennsylvania Press, 1996), 19.
30. John Ernest, *Liberation Historiography: African American Writers and the challenge of History* 1794-1861. (Chapel Hill: UNC Press, 2004), 50.
31. Powell, 101.
32. Ibid., 127.

duties on the streets of Philadelphia were scarcely repaid. A large portion of the *Narrative* dealt with setting the record straight about the common black people's contributions and sacrifices in the face of calamity. The emerging black leadership gave a voice to those whose voice might have been lost to history. A poor black man named Sampson and Sara Bass, who went house to house assisting those smitten by the disease without accepting any payment, were two of the many whose stories were told in Allen and Jones' *Narrative*. [32] Making use of irony, specifically in reference to Carey's allegations of looting the homes of the sick, the *Narrative* discusses an elderly black lady, Mrs. Mabory, whose ring was stolen by the white woman she was tending to when Mrs. Mabory was the first to die. [34]

Beyond this, there is some evidence that blacks, who demonstrated leadership during the epidemic, were catapulted into a greater engagement in the black community after 1793. This is perhaps the most interesting unintended implication of the epidemic. Aside from the intellectual implications of Allen's response, blacks made real gains after the epidemic. In fact, thanks to his efforts during the epidemic, Absalom Jones was propelled into the top leadership position in the African Church of Philadelphia, which in July 1794 was formally connected to the Episcopal church. [35] Gary Nash elaborates on the connection between Jones' rise to prominence and the epidemic:

> "His ministrations to the sick and dying during the terrible days of the yellow fever epidemic had also brought him wide recognition in the black community. 'Administering to the poor sufferers, and soothing the last moments of many departing souls among his people', it was later written, 'he became greatly endeared to the colored race.'" [36]

Also, blacks freed from jail, to help tend to the sick and dead, were able to gain new foothold in a free society and renew identity with the leadership which represented them. The suggestion is not that blacks were able to "prove" their worth to the white Philadelphia as a result of the epidemic, but rather, the event was important as an exercise in black leadership and in shaping the free black society. Of course, these gains came at the expense of many lives, and in another unintended consequence, an immediate emancipation bill that was being considered in the Pennsylvania legislature was not passed as the city had to deal with the disease. [37]

It is true that Richard Allen and Absalom Jones sought to change the white popular opinion of the actions of Philadelphia's black community in their *Narrative*. "The bad consequences many of our colour apprehend from a partial relation of our conduct are, that it will prejudice the minds of the people in general against us," the *Narrative* explains, " [but] we can with certainty assure the public that we have seen more humanity, more real sensibility from the poor coloured than from poor whites." [38] However, Allen and Jones' response represented a vocalization from a dynamic black community that had already started to take form. The formative experience that the epidemic provided the Philadelphia black community to further its development of its leadership and organizational growth.

Not only did Allen anticipate Liberation Theology, but he developed media abolitionism long before it became mainstream in the early to mid 19th century. [39] For the purposes of this discussion, the "liberationist" theology that Allen helped to forge will be defined as an African and African-American interpretation and application of old and new testament texts to the cause of black freedom in the face of American slavery. In particular, the story of the Exodus—Israelites being freed from servitude in Egypt by God— was recast in terms of black bondage and Jesus Christ was characterized as an active servant of the oppressed. While one can certainly look to figures such as Olaudah Equiano for early criticism of the slave trade, Allen's moralist outrage and cognizance of the most volatile theological issues are noteworthy for his time. Equiano, referring to the kidnapping of his sister, asked, "O ye nominal Christians! Might not an African ask you, learned you this from your God? Who says unto you, Do unto all men as you would men should do unto you?" [40] Allen, a Philadelphian, would have been familiar with this Quaker biblical interpretation. [41] He took this interpretation among others, and put them into motion in terms of social vocation in Philadelphia, and the collective spiritual consciousness of Black America.

Blacks had ample reason to distrust the scriptures. Many were introduced to a strictly white interpretation of the Bible: blacks were cast as the descendants of Ham and therefore destined for servitude, and it was said by white preachers that those who wrote the scriptures were white. [42] However, Allen showed surprisingly little anxiety in his methodology. He traced the roots of his faith to a personal transformation and independently developed a Church that was both traditional and revolutionary, working for a social goal. Like many blacks, Allen "made an eschatological decision for black liberation with the 'intuitive knowledge that Jesus Christ had not willed their eternal bondage but their freedom.'" [43] Theologian James H. Cone accurately assessed the role of churches like Allen's AME Bethel Church as serving the "*whole* Black person in the *whole* Society," and outlined the great contributions of the 19th Century Black Church:

> " Led for the most part by illiterate preachers, many of whom were slaves or recently freed, impoverished and repressed by custom and law, this church converted thousands, stabilized the Black family, established insurance and burial societies, founded schools and colleges, commissioned missionaries to the far corners of the world when most Blacks had difficulty buying a ticket on a steamship, *and at the*

33. Allen, 36.
34. Ibid., 37.
35. Nash, *Forging Freedom*, 127.
36. Ibid., 127.
37. Newman *Freedom's Prophet*, 93.
38. Allen, 34.

39. The process that Allen underwent is not unique. Allen Callahan suggests that "slaves and former slaves begin to develop a 'liberationist' reading from the moment they begin to work out their soul salvation under the slave regime." What is significant is the extent to which Allen institutionalized these sentiments giving them the respectability of a theology, church and social organization. Allen D Callahan, *The Talking Book: African Americans and the Bible*. (New Haven: Yale University Press, 2006).

40. Olaudah Equiano. *The Interesting Narrative of the Life of Olaudah Equiano or Gustavus Vassa, the African*. (London: By the Author, 1789), 70. Made available at (http://www.nationalarchives.gov.uk/pathways/blackhistory/rights/docs/equiano_writing.htm)

41. Ibid, 35.

42. Callahan, 53

43. Harry H. Singleton III. *Black Theology and Ideology*. Minnesota: The Liturgical Press, 2002. 9.

> same time, petitioned governments for the abolition of slavery, fomented slave uprisings, organized the Underground Railroad, promoted the Civil War, developed programs of political education and action on behalf of citizenship rights and provided the social, cultural, economic, and political base of the entire African-American community in the United States." [44]

Though Allen never received a formal education, he was literate. While Allen's church was founded in the 18th, not the 19th century, he introduced many of these impacts and served as a model. Perhaps figures like Absalom Jones and James Forten, who both attended Quaker schools, influenced his literary models. He was known to value his erudition and kept a personal library. [45] This, however, in no way detracted from his reputation as a superb and powerful extemporaneous speaker. Such pure vision forced Allen to adhere to a system to personal faith and its community implications. Allen's work during the Yellow Fever epidemic and the work done by the black community mimic these lofty theological abstractions that went undefined until sometime after.

This is the story of a man and a group of oppressed, but hopeful people who engaged in a service that has been long since forgotten to history and the unintended consequences which followed. Beneath what appears to be another instance of blacks being cajoled and forced into an unsanitary labor, one finds an eloquent framing of black leadership, black theology, and black community dynamic. In a way that both parallels and conflicts with the same concepts as we understand them today, the complexities and outcomes of the Yellow Fever epidemic shed light on Allen's genius and places him among the august company of not only the great black leaders, but America's many founders. His narrative ought to be placed alongside the founding documents of protest including the Declaration of Independence, and Paine's *Common Sense*.

Acknowledgement

I would like to thank Larry Hudson for continuous criticism and guidance, as well as Victoria Wolcott for criticism on the original paper. Richard Newman, Professor at Rochester Institute of Technology, also provided invaluable discussion, and assistance in working through available scholarship on Richard Allen. Others who answered questions and contributed feedback include Allen Callahan and Eugene Genovese.

44. James H. Cone and Gayraud S. Wilmore, *Black Theology a documentary history volume one: 1966-1979*. (New York: Orbis Books, 1993), 218.

45. Newman, *Freedom's Prophet*, 113.

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Vapor Deposition of Naphthalene-Derived Polyimides

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Adviser: Mitchell Anthamatten, PhD
Department of Chemical Engineering

In recent history, increasing energy costs are fueling research for a viable cheap energy source. Currently inorganic silicon based solar cells are 10% efficient with a 30 year lifespan. Although they are already in production, cost is still a major issue.[1] A new form of solar cell, organic photovoltaics (OPVs), is being researched. Although inorganic silicon solar cells are currently more efficient than OPVs, the purification process of silicon is energy intensive. OPVs are attractive because they are inexpensive, and require much less energy to produce than silicon.[1] Solar cells have been produced with efficiencies of 5.4%.[2] Many organic photovoltaics degrade easily under atmospheric conditions. Control and stability of film morphology is a key factor in developing efficient OPVs.

As the world energy demand increases exponentially and carbon dioxide emissions increase greenhouse effects, a competitive form of alternative energy must be developed. Many forms of alternative energies have shown to be promising: wind, hydro, bio and solar to name a few. One of the most abundant energy sources on the planet is that of solar energy emitted by the sun. Every day 1.5×10^{22} J of energy reaches the earth, while the approximate human consumption of energy is 1.3×10^{17}J.[10] A specific alternative may not prove to be key to sustaining energy demands. Energy diversity may prove to be as important as biodiversity.

In the world of solar technology, one limiting factor is production cost. As demand for alternative energies increases, the equilibrium market price of solar energy will decrease. But since the base of current solar cells requires highly pure silicon, the production of Si based solar cells may be a more expensive alternative. Organic solar cells do not use silicon substrates and may prove to be an inexpensive form of alternative energy. Current efficiency of research based solar cells for Si based cells is 40.7%[10] and for organic cells 5.4%.[2]

OPVs have been fabricated using several methods including: spin casting, inkjet printing, and chemical vapor deposition (CVD). Solution techniques are attractive because films can be fabricated quickly and inexpensively.[1] However, the use of a solvent can result in film and morphological defects. CVD techniques typically involve evaporation of *p* and *n* type small molecules onto a substrate. Film composition can be controlled by balancing the fluxes of different species. However film stability, phase separation, and crystallization of small molecules can be problematic.[3]

Our laboratory is developing vapor deposition of polyimide materials as an alternative to small molecule deposition techniques. Polyimides are well known to exhibit good thermal and chemical stability. Conjugated polyimides may be useful as n-type semiconductors in photovoltaic devices.[4] To vapor deposit polyimides, reactive precursors can be sublimed and condensed onto a target substrate. There, monomers undergo polymerization to form poly(amic acid), which can be subsequently cured to form polyimide. In our preliminary study we demonstrated that vapor-deposited polyimide can act as a host material for copper phthalocyanine (CuPc), a well known p-type semiconductor. Codeposition of two polyimide precursors, 4,4'-oxydianiline (ODA) and 3,3',4,4'-biphenyl tetracarboxylic (BPDA) with CuPc resulted in polyimide films containing small CuPc crystals. The morphology and crystal behavior were found to depend on deposition conditions.[5]

Here, we explore the deposition of a different polyimide, which is based on naphthalene-tetracarboxylic dianhydride (NTDA) and ODA. Naphthalene diimide small molecules possess conjugated π-electron system high electron mobility (0.1 cm^2/Vs)[6,7] compared to our previously deposited BPDA-ODA polyimides (1×10^{-4} cm^2/Vs).[5] Furthermore we identified a challenge to depositing homogeneous, pinhole free films: monomer crystallization competes with step-growth polymerization. Suggestions are made on how to overcome this issue.

Experimental

Materials: NTDA was obtained from Aldrich at 95% purity. Experiments were performed using both sublimed NTDA and as-received NTDA to assess the importance of monomer purity. Zone refined ODA (98 % pure) was obtained from Aldrich.

Vapor Deposition: Details of vapor deposition are provided in our earlier study.[5] Prior to deposition, all glass substrates were cleaned in an alcohol-water mixture, rinsed thoroughly with

deionized water, and dried under N_2. Reagent crucibles were weighed before and after each deposition. Crucibles were placed in band heaters, and target substrates (microscope glass slides) were affixed to the top of the CVD chamber.

The CVD chamber was brought to a base pressure of 10^{-6} Torr, and crucibles were pre-heated using PID temperature controllers. Previous attempts using fixed temperature evaporators failed to produce a film. Deposition rates of ODA and NTDA were monitored using quartz crystal monitors (QCMs), and temperature was manually adjusted to maintain a constant reagent flux (~10Å/s). Care was taken to eliminate cross reading of rates. When evaporation rates leveled off at a desired rate, the shutter was opened to allow deposition onto glass slides. Rates were monitored throughout the deposition. The shutter was closed at a desired film thickness. Following deposition, films were cured in a nitrogen-purged tube furnace. To do this, films were held at 100 °C for 15 min, 200 °C for 15 min, and 300 °C for one hour.

Characterization: To observe film quality and the formation of crystals, films were studied using polarized optical microscopy, both before and after curing. Films were also deposited on KBr optics, and Fourier-transform infrared spectroscopy (FT-IR) was carried out before and after curing.

Results/Discussion

FT-IR spectra of vapor-deposited NTDA-ODA before and after curing are shown in Figure 2. For the uncured film, peaks are observed for both poly(amic acid) (1738, 1515 and 1497 cm^{-1}) and NTDA crystals (1778,1296 and 1230 cm^{-1}). These peaks disappear upon curing. This is likely due to conversion from poly(amic acid) to polyimide or evaporation of NDTA crystals. Evaporation or polymerization correspond to a decrease in peak height, cf. Figure 2. Upon curing, several new peaks appear that suggest the formation of an NTDA-ODA polyimide. The absorption peaks at 1717 cm^{-1} and 1676 cm^{-1} correspond to symmetric and asymmetric stretch of imide carbonyl groups. The band at 1330 cm^{-1} corresponds to the C-N imide stretch.[8]

Both FT-IR data and optical micrographs indicate the presence of crystals in vapor-deposited poly(amic acid) and polyimide-cured films. Figure 3 shows crystals scattered throughout the material, before and after curing. Crystals appear to have dimensions of ~ 1-5 μm. After curing, the concentration of crystals in polarized optical microscopy significantly decreases, corresponding to a decrease in crystal-assigned peaks (1778, 1296 and 1230cm^{-1}) of the FT-IR. Others have seen crystals in CVD films.[9] A stoichiometric excess of one component may encourage crystal formation. So by adjusting molar fluxes, it may be possible to reduce or encourage crystal growth in CVD films.

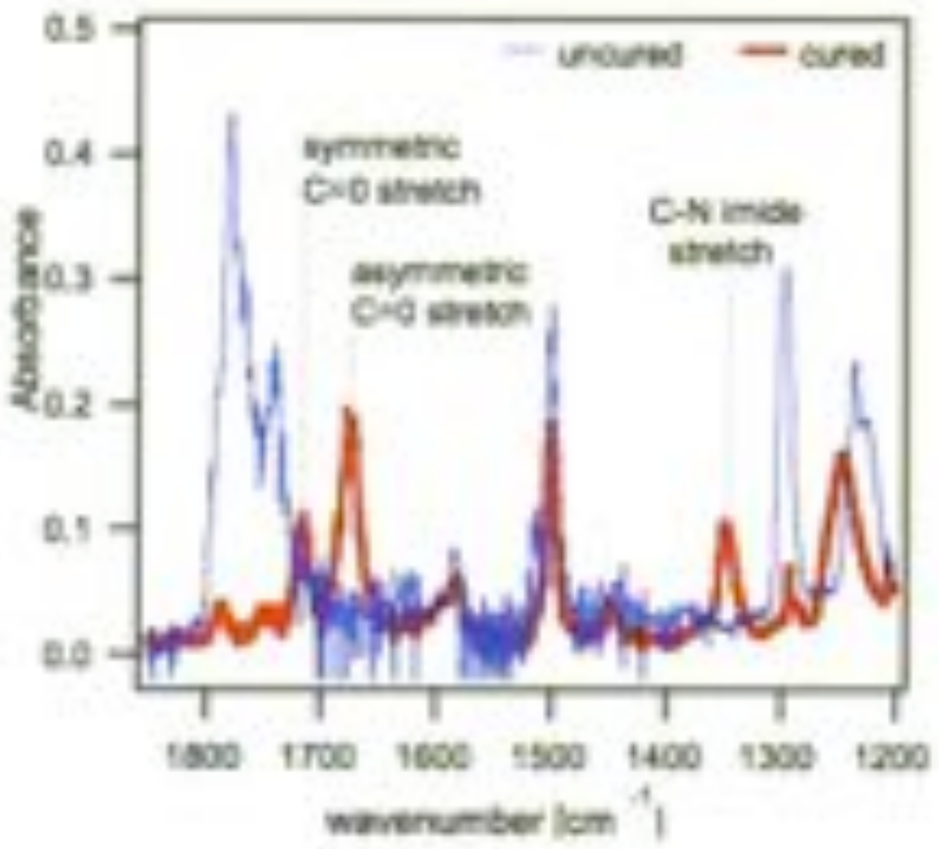

Figure 2. FT-IR spectrum of NTDA-ODA films, both uncured and cured. Polyimide peaks are marked.

Deposition of high quality, crystal-free films requires careful consideration of molar evaporation rates and molar fluxes onto the substrate. Average evaporator molar loss rates, dn_e/dt, were estimated by weighing crucibles before and after deposition. However, these values are estimates, and include mass loss during warm-up and cool-off periods. The average molar deposition rate, dn_d/dt, of each monomer was observed using crystal monitors and was found to scale nearly linearly with dn_e/dt. The mass of monomer deposition onto a QCM and the molar mass loss from a crucible was linked using a proportionality constant (k) resulting in the formula $\frac{dn_d}{dt} = k\frac{dn_e}{dt}$. Values of dn_d/dt and dn_e/dt for several runs are plotted in Figure 4. Indeed, data are linear and each slope, k, represents the fraction of evaporated monomer that is deposited onto the corresponding QCM. Over the range of evaporation rates studied, this fraction is constant for both: (k_{NTDA}=3.8E-03) and (k_{ODA}=2.1E-03).

Conventional knowledge suggests that a stoichiometric (1:1) balance of monomer flux is essential to form homogeneous, uniform films. However, in our study, monomer deposition was assessed two ways: by measuring evaporator mass loss and using quartz crystal monitors. Interestingly, as shown in Figure 5, the

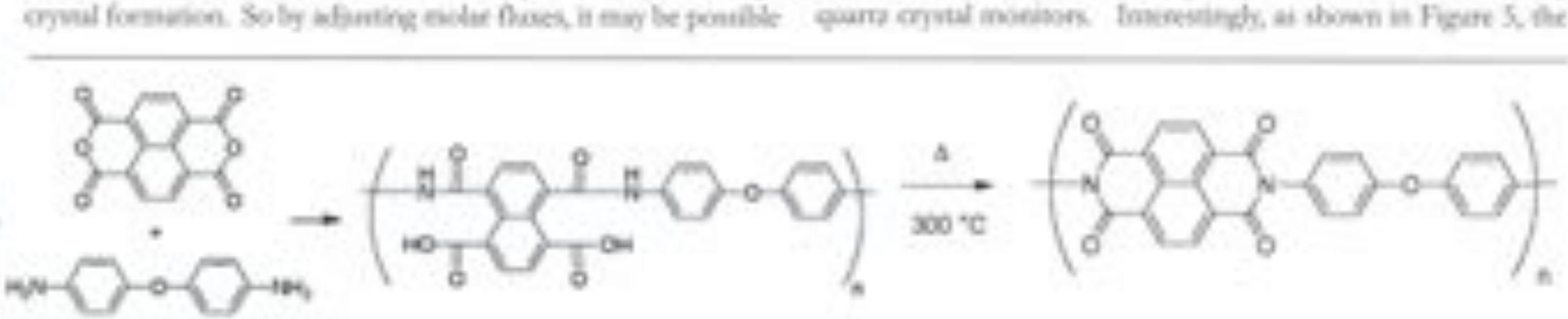

Figure 1. The reaction of NTDA and ODA to form poly(amic acid) followed by curing to form polyimide.

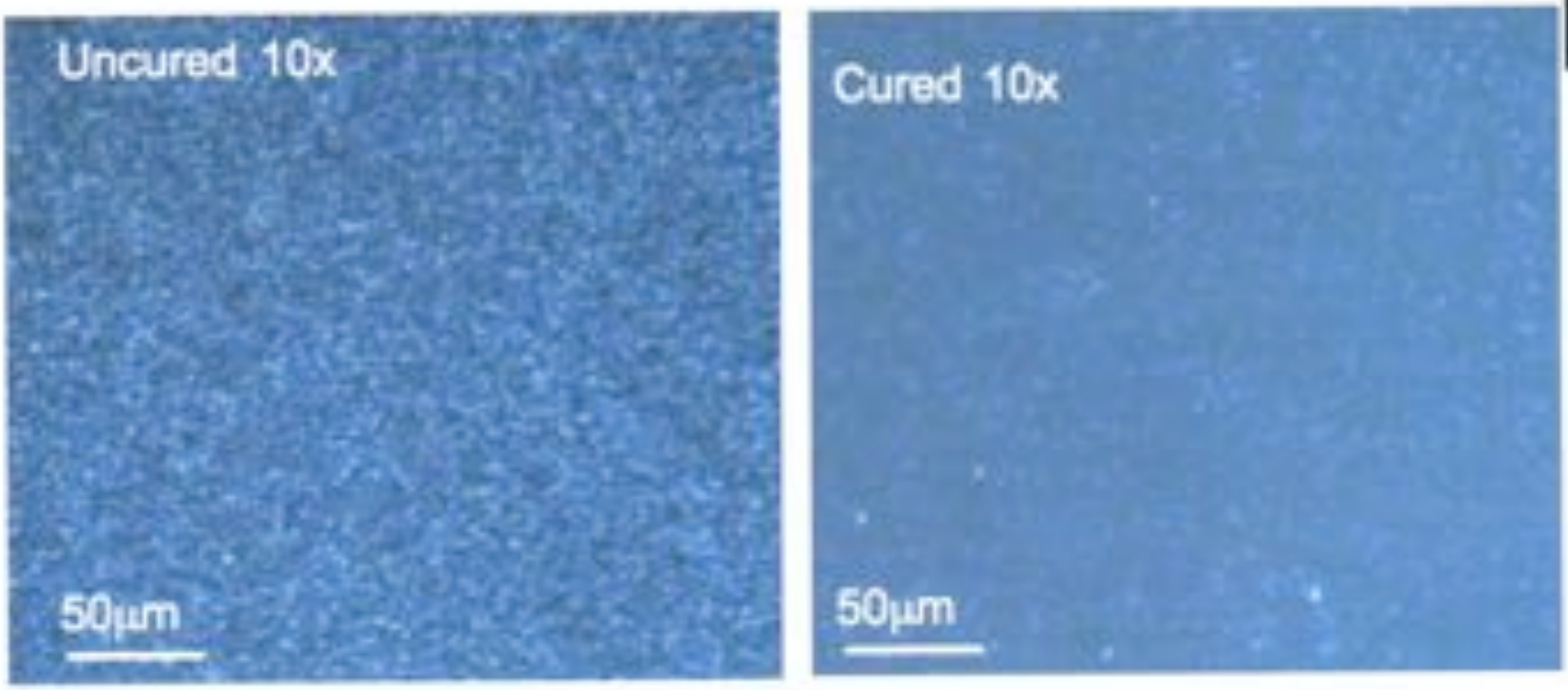

Figure 3. NTDA/ODA films under double polarized light with 10x magnification. From left to right: uncured polyamic acid with crystals, cured polyimide with reduced light scattering

molar ratio calculated using these two techniques does not agree.

Figure 5 compares stoichiometric molar ratio of evaporated monomers (NTDA/ODA) based on the ratio of monomer deposition onto QCMs and the molar loss ratio found by monomer mass loss. The calculated molar loss is a more meaningful because it comes from physical measurement of monomer crucibles before and after each run. The mass of monomer deposition onto a QCM and the molar mass loss from a crucible was linked using a proportionality constant (k) resulting in the formula: $\dot{n}_y = k\dot{m}_y$.

Figure 5 shows a calibration between deposited molar ratios of monomer on QCMs compared to evaporated monomer ratios. The graph shows that the molar ratio of evaporated monomers is proportional to the molar deposition of monomers onto QCMs. Many attempts were made to deposit a film with a 1:1 ratio of monomers. According to the figure, for a 1:1 QCM molar deposition ratio, a molar evaporation ratio of ~2:1 would be needed. It was assumed the crystals formed on films were due to ODA. Monomer charges were then adjusted to increase the amount of NTDA deposited along with ODA. According to FT-IR and polarized optical microscopy, a lower ratio of NTDA/ODA produces less crystalline films.

Future studies will include experiments to better understand how molar evaporation rates are related to crystal growth on QCMs. Also, the position of QCMs with respect to each evaporator may play a significant role in the calculation of deposition ratios.

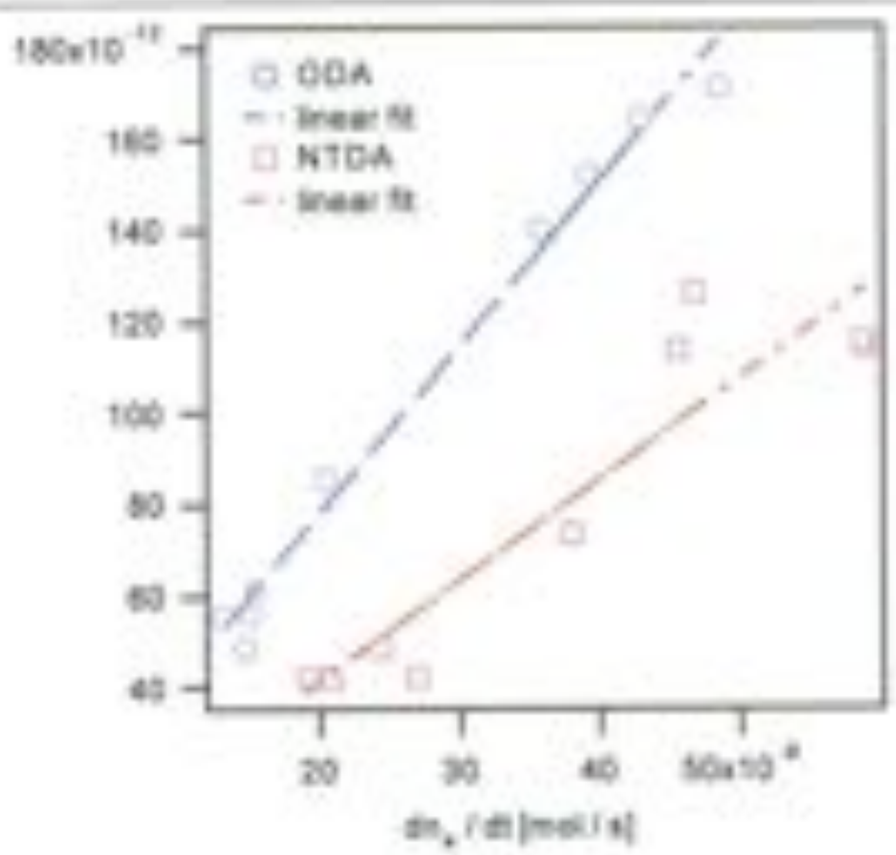

Figure 4. Plot of deposition rate onto QCM monitors vs. monomer evaporation rate from crucibles.

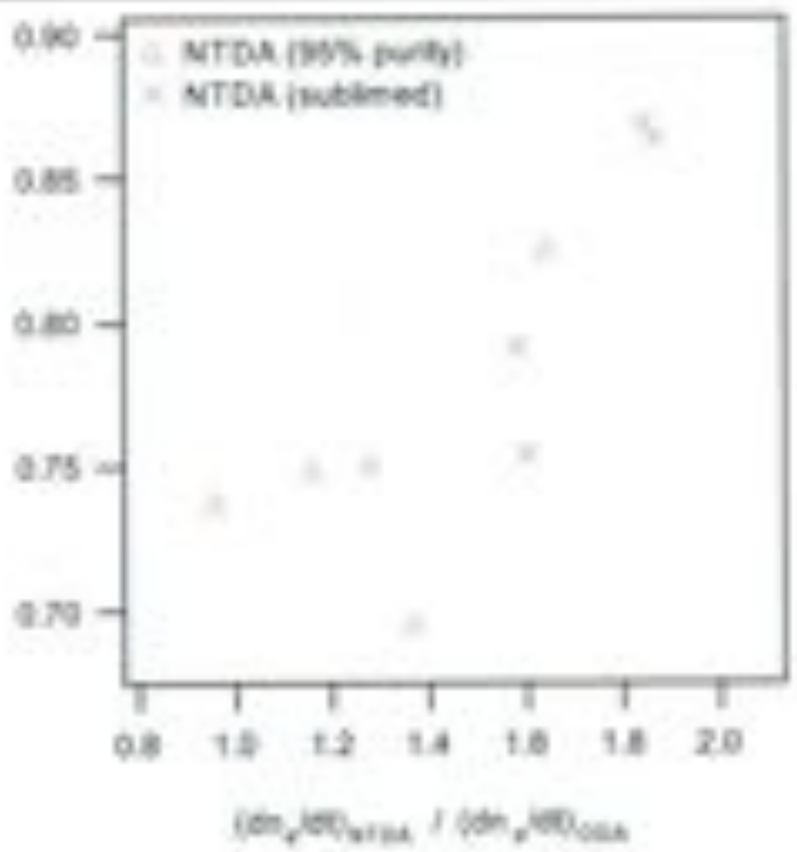

Figure 5. Plot of QCM molar loss ratio (NTDA/ODA) to the calculated molar loss ratio.

Conclusions

In summary, rate-controlled deposition of NTDA and ODA monomers produced a thin film. FT-IR analyses indicate deposited poly(amic acid)s can be subsequently cured to form a polyimide film. Data also suggest that, before curing, a stoichiometric excess of NTDA was present in films. A formula $n_p = k n_o$ and $\frac{dn_p}{dt} = k\frac{dn_o}{dt}$ was written to link thickness of films and evaporated molar mass of monomers by a constant (k). Plots of dn_p/dt vs. dn_o/dt show a linear relationship between molar loss and monomer deposition onto QCMs. The molar ratio calibration curve shows the significance of QCM readings mean in terms of actual molar loss ratios from evaporators. This information can be used in future studies to vary NTDA/ODA molar deposition ratios to produce crystalline reduced films.

Crystalline free films may increase electron mobilities in polyimide n-type semiconductors. Electron mobilities of crystalline reduced films will be tested. Future efforts with crystalline reduced films will incorporate CuPc to produce solar cell devices and their efficiency will be tested. Once shown to be an efficient device, the next step will be to study changes in film morphology to further increase device efficiency. OPVs are still in the research phase. Once higher efficiency devices can be shown to work, with sufficient durability, they can become a competitive alternative to Si based solar cells. OPVs may be a successful competitor to current Si based solar cells.

Acknowledgement

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Student Perspective

Xiaoge Guo

Cancer Treatment: Still Finding the Magic Bullet

"We must search for magic bullets. We must strike the parasite and the parasite only, if possible, and to do this, we must learn to aim with chemical substance."

-Paul Ehrlich, Father of Chemotherapy

The argument for the effectiveness of chemotherapy whether it can prolong life has been going for a while. Scientists and doctors have constantly been coming up with new drugs that can help combat cancer cells. But over time, people have expressed frustration and started to question whether the tremendous amount of funds poured into the research for anti-cancer drug will eventually have a fruitful outcome. How serious are we in terms of finding a cure for cancer?

Based on the data provided by CDC (Center for Disease Control), cancer is the second leading cause of death in the US. Each year, 1 M cancer patients undergo chemotherapy. Along the treatment line, these 1 M people suffered from the dreaded side effects of chemo such as vomiting, nausea, and hair loss. After the treatment, patients always find it hard to have any food intake due to difficulty in swallowing. All these side effects however are signs of the chemo drug doing its job. The drug targets not only the rebellious cancer cells dividing uncontrollably but also healthy cells that normally would divide rapidly such as hair cells. At this point, chemotherapy might help our body system get rid of cancer cells but at great cost. The question remains: should chemotherapy still be recommended and should the million dollar research keep going on?

In recent years, chemotherapy has forwarded into monoclonal antibody and hormone therapy. On a molecular level of treating cancer, monoclonal antibodies bind to cancer cell-specific antigens and induce immune response to target cells. Hormone therapy involves taking medications that interfere with hormone activity or having glands to be surgically removed during the treatment. As all drugs go, these two treatments also have severe side effects. However, just recently, FDA just approved a patch called Sancuso that can be worn on patients who just went through chemotherapy aiming to remove the nausea and vomiting resulted from the treatment. Sancuso is a medicine that gets into the body through the skin. This patch is expected to be offered to the public by the end of the year. Other drugs that address the side effects are also available.

When chemotherapy was first started in practice in the 1940's, the goal in the science world was to be able to find the magic bullet that will shoot at the target specifically and directly as Paul Ehrlich the founder of chemotherapy had hoped. After two decades of research, scientists have still yet to fully uncover the complexity of cancer pathways. While cancer cells are constantly evolving new mechanisms to survive drugs, scientists are also brainstorming to find novel drugs to battle cancer cells. Chemotherapy may not be the answer but it can serve as an answer to cancer before the magic bullet is still to be discovered.

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The Effect of Mechanical Loading and Hydrogel Structure on the Release of ECM Components

Suzanne Giunta, 2010
Advisers: Dr. Stephanie Bryant and Garret Nicodemus
Department of Biomedical Engineering

Walking, running, and standing are all actions that a typical person takes for granted on a daily basis, yet even one microscopic tear in articular cartilage can put an end to all of these activities. Tissue engineering is a promising field for the regeneration of cartilage, but functional cartilage has yet to be produced. This project lies in the field of cartilage regeneration and specifically focuses on the use of mechanical stimulation to encourage chondrocytes to produce functional cartilage tissue. Dynamic compression in vitro seems to be a promising way to mimic natural stress that the body places on articular cartilage on a daily basis and a few studies have supported the hypothesis that chondrocytes (cartilage cells) benefit from mechanical loading.[1] However, little is known about how to use mechanical stimulation to optimize tissue growth and minimize tissue degradation.[2]

A problem encountered in this research is the release of cartilage components, which make up the essential extracellular matrix (ECM) from poly(ethylene glycol) (PEG)-based hydrogels while in culture. To examine this problem, two common ECM components were loaded in PEG hydrogels in the presence and absence of mechanical loading. Specifically, chondroitin sulfate (ChS) and hyaluronic acid (HA) were used as model ECM components. Chondroitin sulfate is 25 to 30 disaccharide units long and is one of two types of glycosaminoglycans (GAGs) that covalently attach to a protoglycan (PG) core, forming larger structural macromolecules, such as aggrecan[3]. Aggrecan is then physically bound via a link protein to a specific binding region on a much longer hyaluronan molecule (100-4,000 kDa). The complete PG can reach a MW of 20-100 million spanning micrometers in length, depending on the GAG and PG composition. This forms 5-10 % of the ECM, while another 15 % is composed of a dense network of fibrous collagen (mostly type II) and the other ~80 % is water, with a sparse population of chondrocytes. This water is subject to move in and out of the ECM due to concentration and/or pressure gradient changes.[3]

Poly(ethylene glycol), or PEG, hydrogels were used to encapsulate the components of the ECM that were studied for their release because of their biocompatibility with tissue engineering scaffolds. The hydrogels are insoluble, but their hydrophilic nature allows them to swell when immersed in aqueous solutions. This allows for the diffusion of biologically necessary particles such as oxygen and nutrients into and out of the hydrogel, which can be studied. PEG hydrogels' properties can also be easily manipulated, such as cross-linking density and degradation rate, which combined with the fact that they are compatible with cells such as chondrocytes, makes PEG hydrogels a good candidate for creating scaffolds.[4]

Our PEG hydrogels were formed via photopolymerization, a process that creates hydrogels by a radical chain reaction, and via REDOX reaction, a process in which a chemical reaction takes place to turn the liquid macromer solution into a solid hydrogel. The photoinitiation reaction begins when an initiator absorbs light at a specific frequency and reacts to form radicals, which interact with the vinyl groups in PEG to begin the chain polymerization reaction. The photoinitiator used in this study is the UV-photoinitiator (1-[4-(2-hydroxyethoxy)-phenyl]-2-hydroxy-2-methyl-1-propane-1-one), or Irgacure 2959, (Ciba-Geigy) because of its ability to dissolve in water. The choice of photoinitiator is important because it controls the rate at which the gel polymerizes and plays a major role in determining the final gel structure.[4]

Characterization of the hydrogels that were created for their mechanical properties is important to be sure that all gels created exhibit the properties that are predicted. One important property to characterize in gels is known as the equilibrium swelling ratio, or Q, which expresses how much the gels swell. The more PEG that is added to the gels, the higher the cross-linking density and lower the amount of water that is able to diffuse into the hydrogel, which is important for cell survival. Q can be calculated from the ratio of the weight of the swollen gel, Vs, to the dry gel, Vd.

$$Q = \frac{V_s}{V_d} = 1 + \frac{\rho_p}{\rho_s}(q-1), \quad q = \frac{M_s}{M_d} \qquad (1)$$

The cross-linking density (ρ_p), which described how much PEG macromer is in the gel, is also important to quantify, as it determines how much water can fit into the gel and various mechanical parameters, such as the compressive modulus.[4] The cross-linking density can be determined by:

$$\frac{1}{\overline{M}_c} = \frac{2}{\overline{M}_n} - \frac{\left[\left(\frac{v}{v_0}\right) + \ln\left(1 - v_{2,s}\right) + \chi_1 v_{2,s} + \chi_1 v_{2,s}^2\right]}{v^{1/3}_{2,s} - \frac{v_{2,s}}{2}} \quad (2)$$

The final gel parameter that was calculated was the mesh size, which represents the space, in angstroms, between two polymer cross-links that are next to each other. This is important when predicting what solute particles will be able to move in or out of the hydrogel.[4] This is calculated by:

$$\xi = v_{2,s}^{-1/3} C^{1/2} n_b^{1/2} \quad (3)$$

Using this background information of cartilage components and hydrogel structure and formation, our goals were three-fold. First, examine the effects of cross-linking density (ρ_c) on the release of chondroitin sulfate (ChS) and hyaluronic acid (HA) from free – swelling PEG hydrogels. We predicted that increasing ρ_c will result in a slower, sustained release of ChS and HA through decreases in gel porosity. Second, examine how mechanical loading affects gels at different cross-linking densities, especially as a function of frequency. For this, we hypothesized that dynamically loading the gels will increase fluid flow within the gels, resulting in increased release of ECM components. Lastly, once the effect of cross-linking density and mechanical loading on the release of ECM components has been explored, we will include a peptide from link protein (LP) in the hydrogel to determine if it can bind HA to the gel and hinder the release of HA from the gel. If the peptide is able to bind HA to the mono-acrylated PEG, the biomimetic moiety of peptide will decrease the release of HA.

Methods

1. *Hydrogel formation – by photoinitiator*

Poly(ethylene glycol) dimethacrylate (PEGDM) gels with molecular weight (MW) 3000 g/mol were fabricated at varying cross-linking densities by varying the macromer wt.% in PBS so that it was 10, 15, and 20%.

Components used in loading experiments were dissolved prior to gelation at the following concentrations: ChS (50 mg/g), fluorescently labeled hyaluronic acid[5] (f-HA) (1 mg/g), and Link Peptide[5] (LP) (1 mg/g). For photopolymerization, the photoinitiator Irgacure 2959 (1-[4-(2-hydroxyethoxy)-phenyl]-2-hydroxy-2-methyl-1-propane-1-one) (Ciba-Geigy) was used at concentrations of 0.05, 0.025, 0.0125 % for different wt.% gels, respectively.

- The following graph displays the exact amounts of hydrogel components that were added to create the stock gel solution:

Ingredients (µL)	**10% PEG gels**	**15% PEG gels**	**20% PEG gels**
PEG-DM stock	183	275	367
Photo-initiator (12959)	45.8	20.4	11.5
PBS	321.2	254.65	171.5

- ChS was also added to all gels. 27.5 mg was added to all gels except for the 20 % PEG gel combination of ChS/HA/peptide, in which case 60 mg was added.
- Once a stock solution is made for each gel, 85 ul. was pipetted into prepared cylindrical gel molds and placed in a UV light (~6 mW/cm2) for 10 min. These gels were then rinsed with 2 mL of DI-H20 and placed on the loading machine within 12 hours.

Hydrogel formation – by REDOX reaction

For REDOX initiated polymerization, Ammonium persulfate (0.05 M) and TEMED (0.05 M) were used instead of 12959, so the photoinitiator would not interfere with the fluorescently labeled HA.

- The following graph displays the exact amounts of hydrogel components that were added to create the stock gel solution:

Ingredients (µL)	**10% PEG gels**	**20% PEG gels**
PEG-DM stock	200	400
HA (mg)	0.73	0.59
PBS	340	140
Ammonium Persulfate (1 M 1:20)	30	30

- Combine all of the above ingredients and mix 80.25 µL of solution directly into prepared pipettes along with 4.75 µL of TEMED.

Cylindrical gels were formed (5x5mm) at room temperature and hidden from light for 15 minutes. Gels were then immediately rinsed with 2 mL of DI-H20 to collect any soluble fraction and prepared for immediate loading conditions.

2. *Hydrogel characterization*

Swollen wet weights were collected prior to freeze-drying. Subsequently, dry weights were also measured to determine the swelling ratio (Q). Q was used to calculate cross-linking density (ρ_c) and mesh size.[4]

3. *Hydrogel testing*

All gels tested under loading regimes were tested at 15 % strain. Gels were either individually placed into a BOSE Material Tester and subjected to unconfined compression under permeable platens, which had a large enough mesh size so that ECM components that were released could permeate, or loaded into a custom-built compressive loading device[6] (Knee) and also subjected to similar unconfined compression. All samples were cultured in 2 mL of DI H2O.

Periodic samples were taken by stopping the machine, taking the solvent, then re-loading the gel with 2 mL of fresh water. The free swelling samples were tested concurrently with the loaded samples.

4. *Assays*

Two assays for detecting sulfated glycosaminoglycans (GAGs) were tested in the first week to determine the best method for

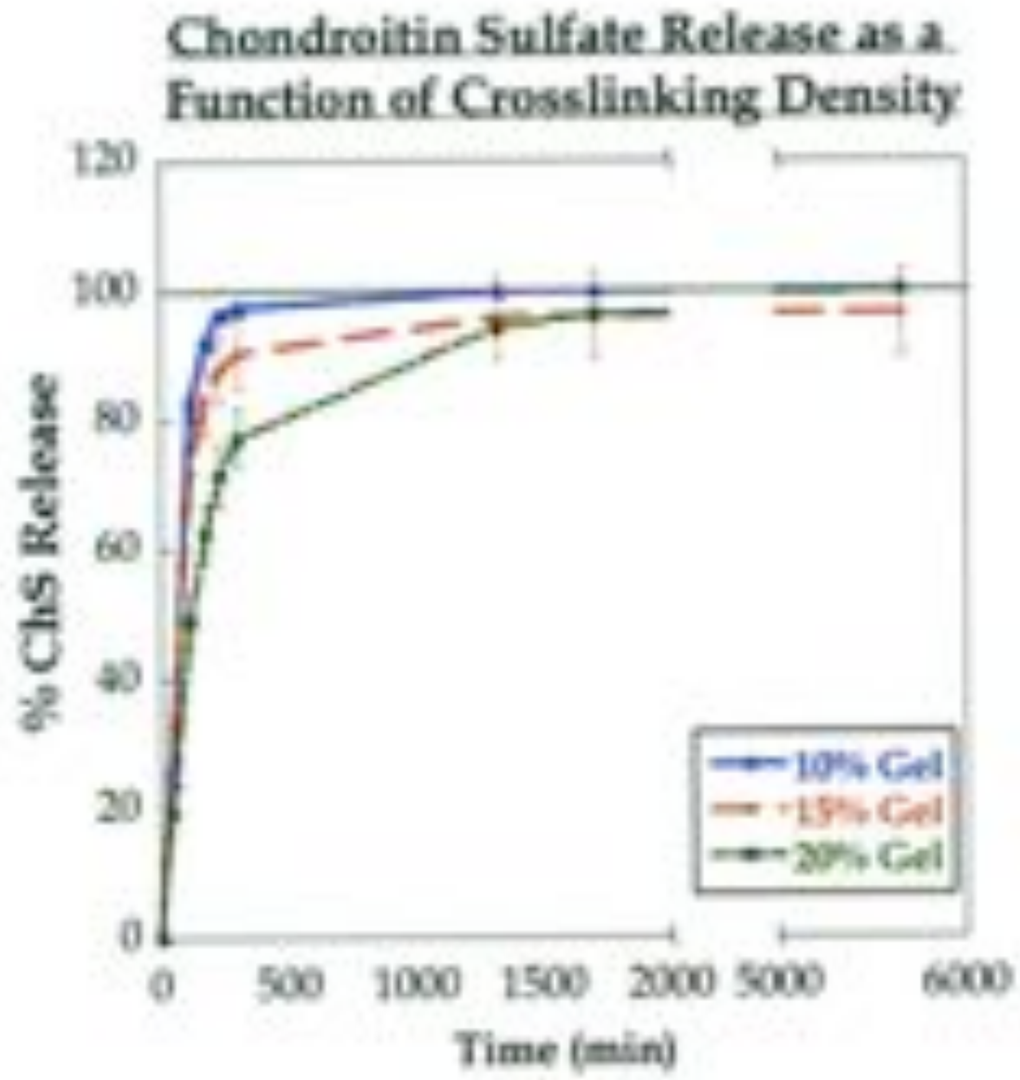


Figure 1.

quantifying the amount of ChS in the supernatant surrounding the hydrogels at give time points.

A modified Dimethylmethylene Blue (DMMB) assay was used to determine the amount of ChS released from gels. The first time though, only one wavelength was used to detect the dye-ChS molecule, but we determined that using two different wavelengths of 530 nm and 590 nm to detect the dye-ChS molecule would give more accurate results for detection of GAGs at concentrations from 10- 100 μg/mL, and the latter method was used for all assays.[7]

Another method of detecting GAGs was also tested. This method, from Barbosa et al, 2003 was tried because of its higher sensitivity to quantifying GAGs by using a complex solution to bind to the ChS, which comprises GAG, than a decomplex solution to dissolve the dye-ChS precipitate. This method is more accurate because there is no precipitate to interfere with the spectrophotometer's readings and was shown to detect concentrations of GAG from 0.5 – 80 μg/mL.[8] However, the concentration amounts that were released by our gels were high enough that the former assay was more appropriate, and it was used to quantify the amount of ChS.

For detection of released 5-FAA, samples were compared to a standard curve measured at an excitation of 490 nm and emission of 520 nm using a florescent setting on the spectrophotometer.[9]

Results, Discussion, and Recommendations

The first objective of our study was to examine the effects of cross-linking density (ρ_x) on the release of chondroitin sulfate (ChS) and hyaluronic acid (HA) from free – swelling PEG hydrogels. This was done by creating PEG hydrogels at 10, 15, and 20 % weight percent and studying their release of ChS over time. The first study was done over a period of roughly 100 hours to get an initial idea of how quickly the hydrogels released ChS. As seen in Figure 1, about 80 % release of ChS was observed within the first 2 hours for the 10 % and 15 % PEG gels, with a 95 % release within 4 hours.

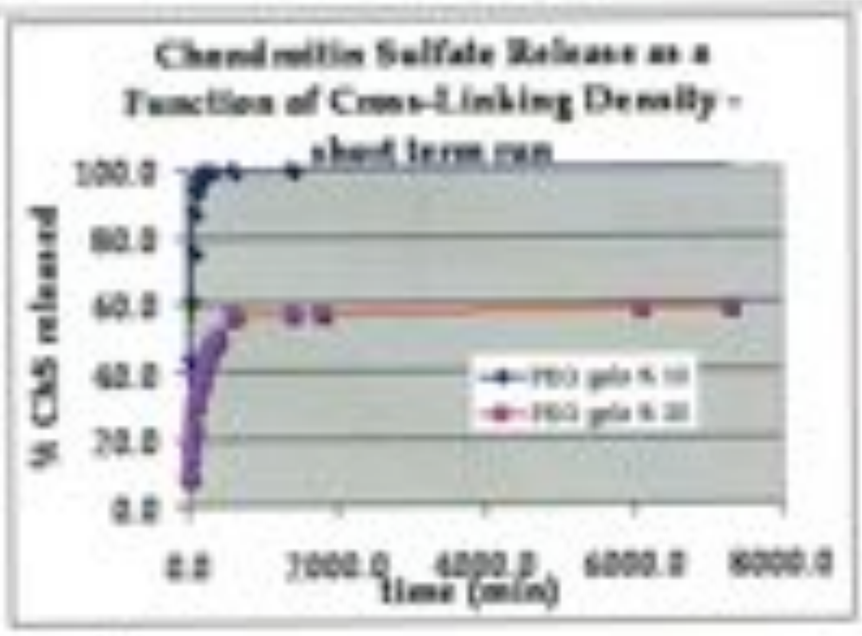


Figure 2.

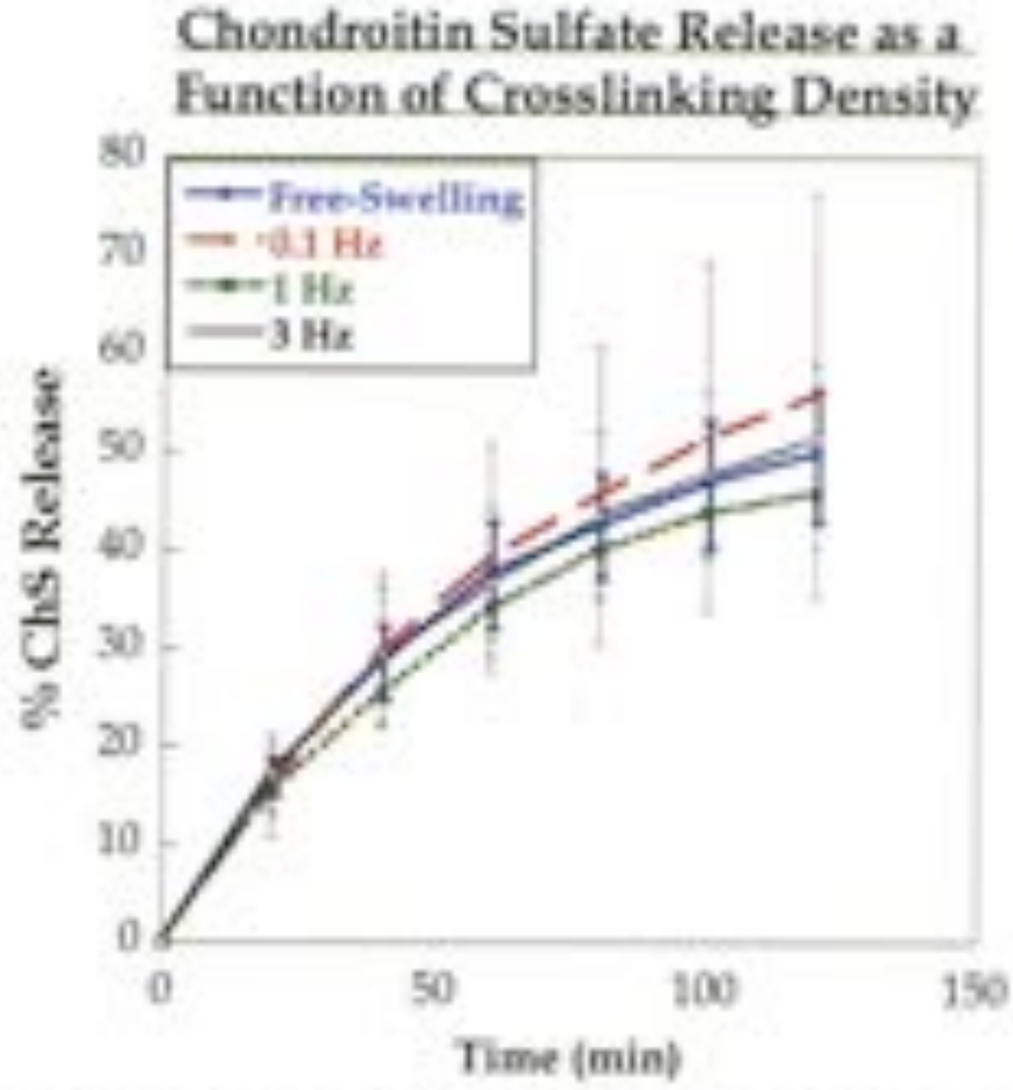
After the first run to get an idea for how quickly the hydrogels were releasing ChS, another study was run over a span of 122 hours, but with time points taken every 20 min for the first 7 hours to get a better idea of how the hydrogels were initially losing ChS. The study was still carried out over 100 hours to be sure that the same equilibrium trend lines that were seen in the first study were noted again. See Figure 2.

As Figure 2 shows, the curves that resulted from graphing % ChS release over time look very similar to the ones shown in Figure 1, when fewer time points were taken, though an even more noticeable rate of release is noticed between the 10% and 20% weight gels after about 500 minutes. This helps to confirm the release trends of ChS from our hydrogels observed with the first study. The nice part of the more sensitive study though, is that the difference in release between the different weight percents of PEG is more distinguishable. As seen in Figure 2, the 10% gels released faster and the 20% gels had the slowest release rate. The faster release in the 10% gels is most likely due to the decreased cross-linking density and increase in mesh size of the gels. As the cross-linking density increases, the more PEG there is in the gels, and since the gels are all the same size, the less room there is for molecules such as water. This decrease in the water content, as seen in the gel characterization data of Figure 3, leads to decreased fluid flow in and out of the hydrogels, which leads to a slower release of ChS from the hydrogels.

For these studies, we characterized the hydrogels as described in the methods section, for Q, mesh size, and cross-linking density. See Figure 3.

PEG wt.%	Time (hr)	Q	Mesh Size (Å)	ρ_x (mol/L)
10	2	14.4± 1.2	195 ± 19	7.30E-02 ± 1.13E-02
	140	11.6 ± 0.2	148 ± 3	1.08E-01 ± 3.72E-03
15	140	8.4 ± 0.1	94 ± 2	2.15E-01 ± 5.34E-02
20	2	7.2 ± 0.3	77 ± 3	2.96E-01 ± 1.81E-02
	140	6.5 ± 0.2	65 ± 2	3.82E-01± 2.06E-02

Figure 3.



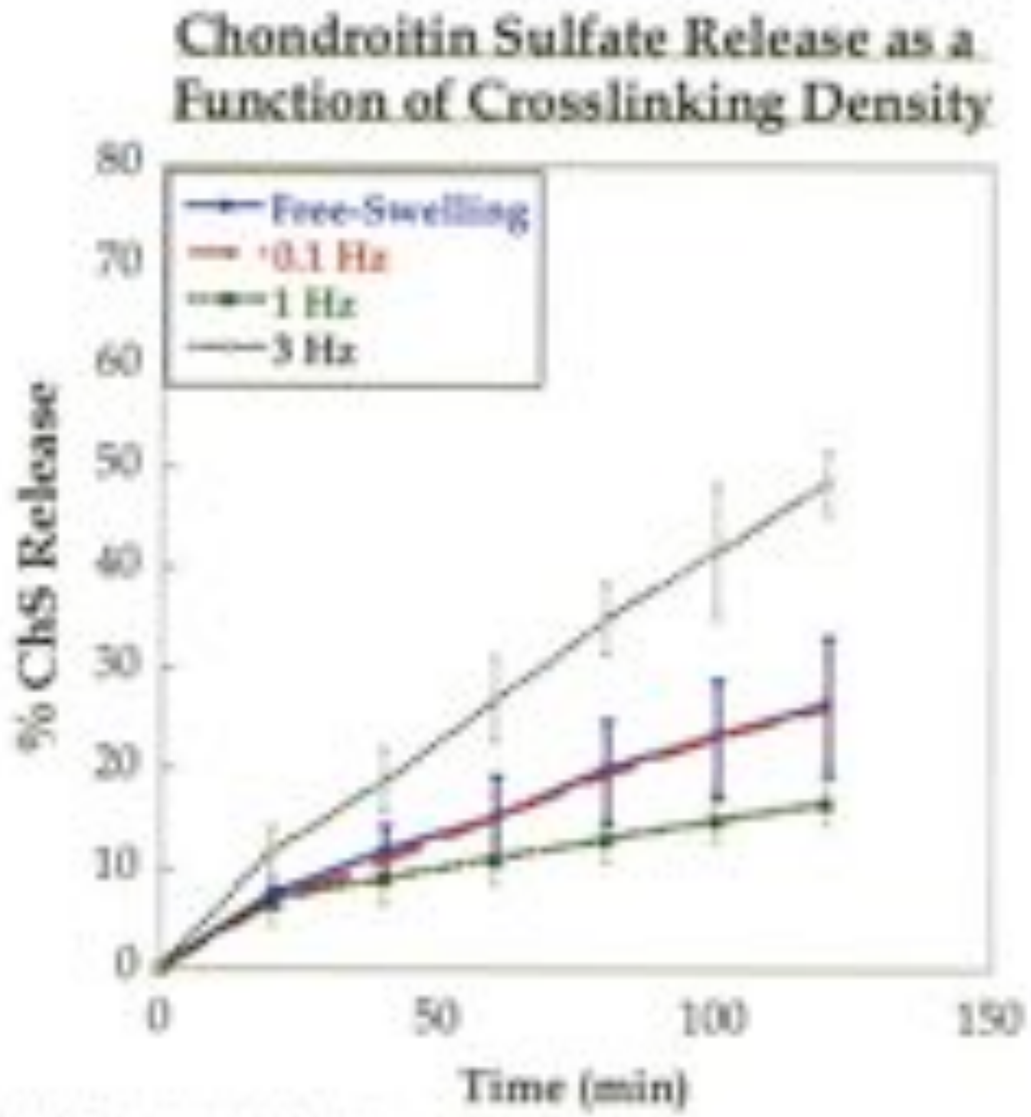


Figure 4 (left). Represents the samples that were made with a 10 % PEGmonomer solution.
Figure 5 (right). Shows the same experiment but with 20 % weight PEG.

As mentioned above, significant differences can be seen between the gels as a function of weight percentage, as well as of time. Q, or volumetric swelling ratio, is doubled as the weight percentage was halved, as seen in the 10 and 20 % weight PEG gels for the 2 hour time points, due to the increased water in the 10 % weight gels. The mesh size, being a function of Q, also changed significantly for the different weight percentages, due to the amount of macromer per the same volume of gels. Cross-linking density (ρ_c) does not vary linearly with the amount of PEG per weight percent, though, as the difference in ρ_c more than triples between the 10 and 20 %. This phenomena is also observed in other literature, which predicts that the ρ_c will increase by a factor of 3 from the 10 to 20 % and cites this due to cyclization of the pendant double bonds of the macromer, as they link back on themselves instead of forming a cross-link with another kinetic chain.[4]

It is also interesting to note that the Q value for a given weight percentage varies over time, as it decreases over the course of 140 hours. This is most likely due to the loss of ChS over time in the hydrogels; as the ChS leaves the hydrogel, the gel becomes more positively charged, while the ChS chains are slightly negatively charged. As the negative charge leaves the gels, there is less of a pull of water into the gel, so the gel will decrease in swelling.

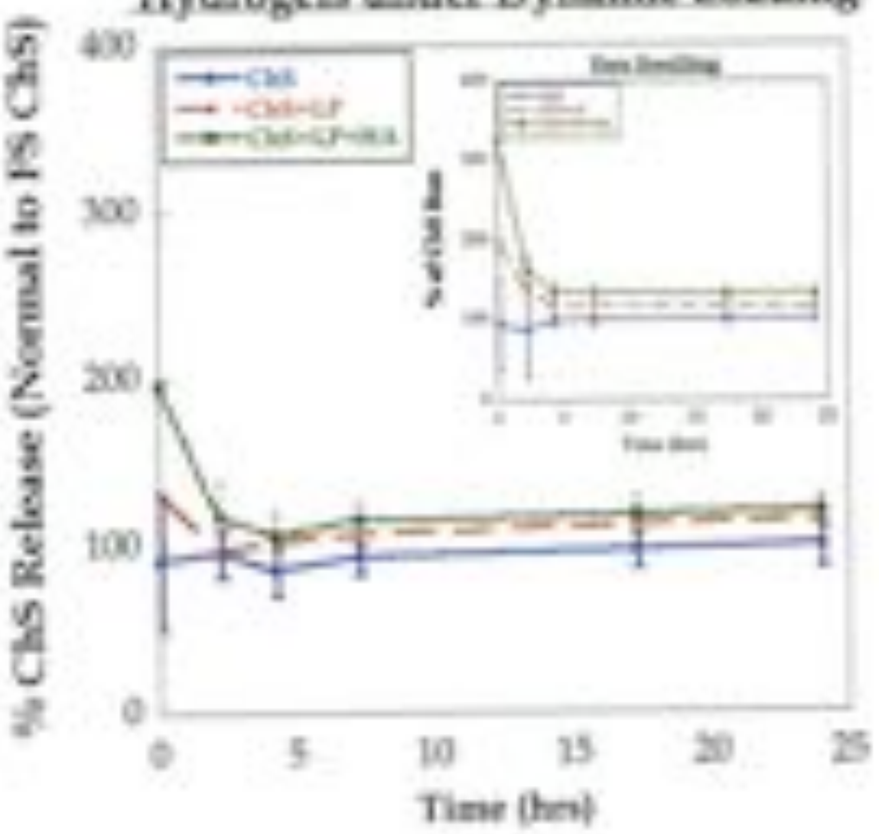
Hydrogels were also attempted to be made with f-HA as the ECM component to be studied for release. However, the gels were created using photoinitiation and the fluorescently-labeled part of HA interfered with the polymerization process. Furthermore, we observed a 50 % initial release of HA from the gels, which means that only 50 % of the f-HA was entrapped in the gel, so the results were not accurate.

After the effect of one variable, cross-linking density, was observed on the release of ChS, another variable, mechanical loading, was introduced to see its effect on the release of ChS. 10 % and 20 % gels were dynamically loaded at 0.1, 1, and 3 Hz frequencies and then compared to concurrently-tested free-swelling samples. Each sample was made and tested 3 times to produce more accurate results.

Introducing the added variable of dynamic loading added another interesting parameter affecting the release of ChS. The 10 % gels did not have significant statistical difference, no matter whether they were free swelling or loaded at varying frequencies. This is most likely due to the quick release of the 10 % gels. The ChS can escape relatively quickly due to the related increased Q, decreased cross-linking density and increased mesh size. These structural characteristics of the gel seem to play more of a role in determining the rate of release of ChS than loading parameters. Future work could be done to carry this experiment out over the course of a day or more to see if the trend lines continue in a similar fashion, given more time to allow for more components to diffuse out.

A greater effect of loading the gels was seen on the release of ChS in the 20 weight % gels. The difference in release due to frequency is observed in the higher crosslinked gels due to the slower sustained release, most likely due to a lower water content and significant decrease in mesh size.

As predicted, the 3 Hz loading frequency gels saw a faster release (~50 % release) than the rest of the gels. This is most likely due to the increased rate of fluid flow in and out of the gels that occurs when a faster loading regime is implemented. The free-swelling and 0.1 Hz samples were seen to release at about the same rate. A possible explanation for this phenomenon could be that the frequency of 0.1 Hz loading is so slow that it is close to not dynamically loading the samples, and therefore, does not create a great increase in fluid flow in and out of the gel. Yet mechanical loading still does have some give and take on the gel so as not to continuously put stress on the gel, as would be seen in statically loading the gels, which has been seen to actually decrease the rate



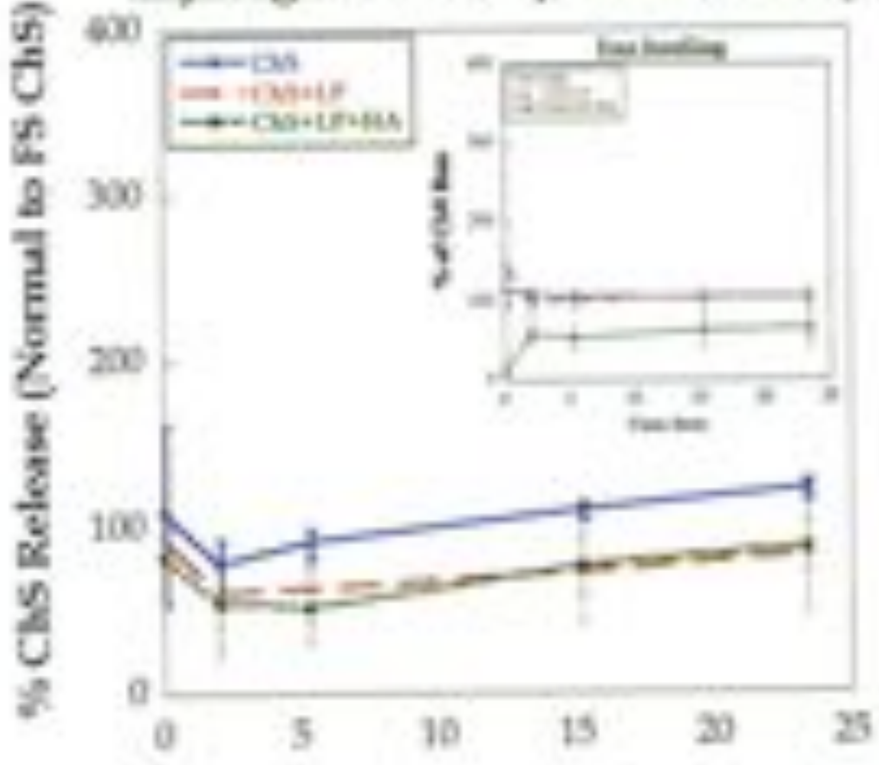


Figure 6. (left) and **Figure 7.** (right)

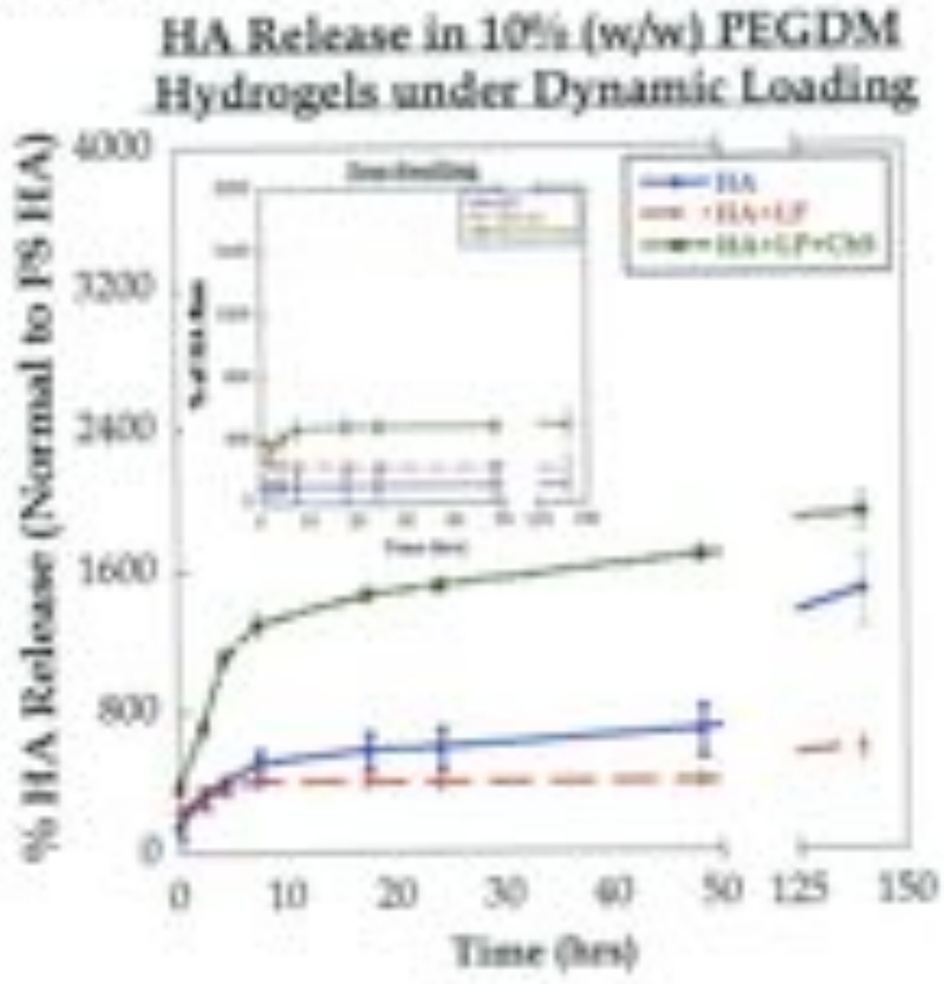
of release of ECM components.[3] This combination of very slow fluid flow due to loading and give and take on the gel is probably the right combination to produce similar release trend lines to that of the free-swelling gels.

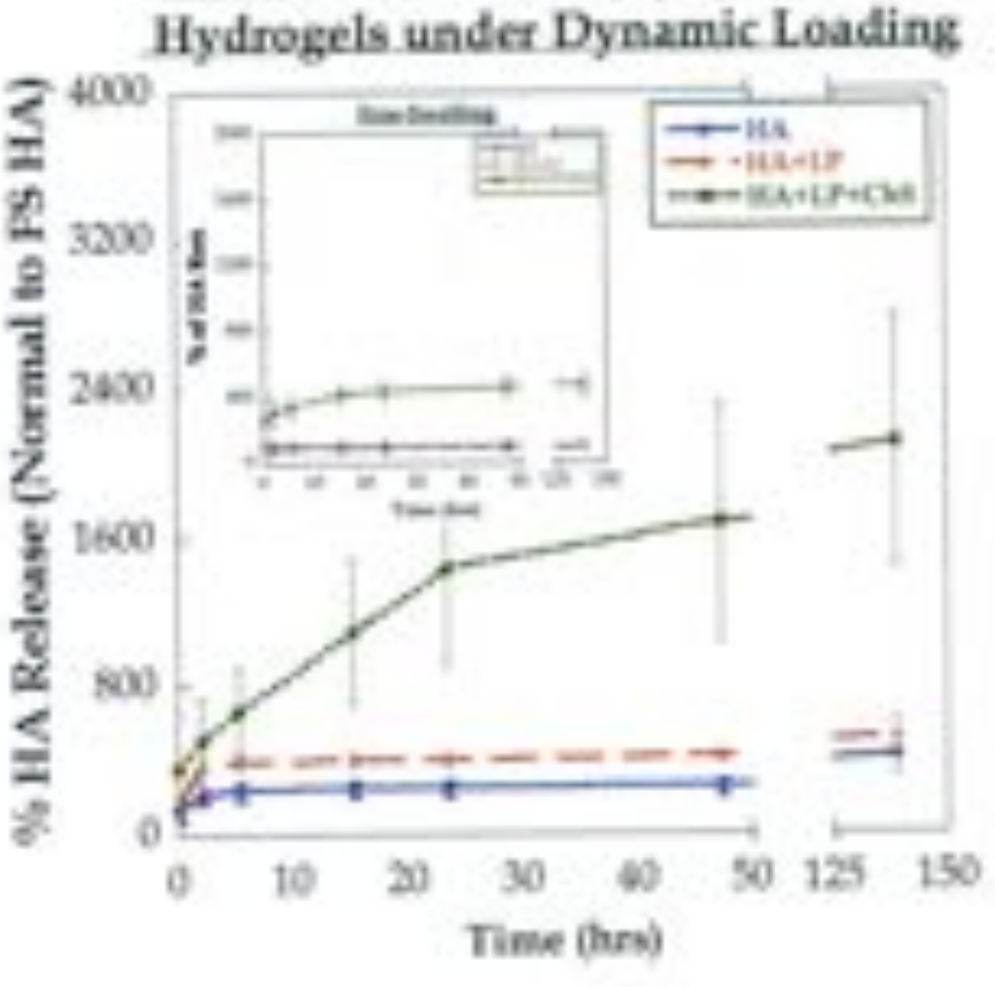
The most interesting data is represented in the lowest lying line of Figure 5, which shows mechanical loading at 1 Hz frequency. This result was surprising, because we predicted that the line would lie somewhere between the 0.1 Hz and 3 Hz lines. However, once these trend lines were noted, the 1 Hz samples were re-run only to produce similar results to the first run. We do not have a feasible explanation for these results but it is interesting to note that the 1 Hz frequency is the same natural biological frequency of loading that articular cartilage experiences when a human is moving at a walking pace. Future work is needed to explore other physiological, structural, or mechanical phenomena that could be causing the decrease in rate of release of ChS from 20 % weight PEG hydrogels when dynamically loaded at 15 % strain at a frequency of 1 Hz.

The last part of this study was to incorporate a link protein (LP) into the hydrogel along with ChS and study the release of the HA and ChS in various combinations of all 3 ECM components. Theoretically, the link protein, or peptide, should bind the HA to the PEG macromer and there should be a noted decrease in the rate of release of HA, as compared to the free-swelling gel that only contains HA. The results from this final experiment can be seen in Figures 6-9.

Figures 6 and 7 display the results of the final study, as measured by the release of ChS from hydrogels that were loaded with just ChS, ChS/LP, and ChS/LP/HA, both loaded and free swelling. In the 10 % gels, other than large initial release in the ChS/LP/HA gels, the gels all had very similar release profiles of ChS over time. In the higher cross-linked gels, the free swelling samples show that the ChS/LP/HA combination varies significantly from

Figure 8. (left) and **Figure 9.** (right)





the other gels, possibly due to interactions caused by the presence of HA. The loaded samples, meanwhile, see more release of ChS in the ChS-only gels, while decreases in release due to loading in both other conditions were observed. The decrease in the release of ChS over time could be due to the increase in the components initially put in the hydrogels. The addition of LP and HA to the gels could be hindering the release rate of ChS due to particle collision when ChS tries to leave the hydrogel.

The synthesis of these gels comprised of HA went much better than the first time, as the gel polymerized instead of forming a soft/partially still liquid gel. This is because we changed our method of gel polymerization from photoinitiation to REDOX reaction. The REDOX reaction does not involve a UV photoinitiator, so there is no interference of the f-HA with the polymerization process, and the gels were able to solidify.

As seen in Figure 8, there is a more noticeable difference in the release over time of HA, as compared to ChS. Significantly more release of HA is observed when combined with LP, which is heightened in the presence of ChS. Dynamic loading results in 400-2000% greater release of HA compared to free-swelling. Under loading, the sole presence of LP appears to reduce the release of HA, whereas ChS increases the release drastically. The results of HA/LP make sense, asserting that the LP physically binds HA to PEG, thereby entrapping it within the gel. And the initial large release of HA/LP/ChS in both the free-swelling and loaded samples becomes smaller over time, as the HA loaded gel dramatically increases after 130 hours.

Under gels of higher cross-linking, no change in release was seen with the addition of LP. However, ChS addition resulted in ~400% greater release over time. Under loading, the release of HA reached 400% of that in FS samples, and ~2000% when both ChS and LP was added. The noticeable difference in the release of HA that was noticed in gels of both weight percentages could be explained by the fact that ChS causes an increase in the swelling of the gel. The negative charge on ChS could be pulling more water in, thereby increasing the fluid pulled into and out of the gel. The added variable of mechanical loading also facilitates fluid flow which would be conducive to more opportunities for the HA to leave the hydrogel.

It should also be noted that the data on this experiment's graphs have all been normalized to free-swelling HA, as opposed to being normalized to free-swelling gels that have the same components added to them. This means that the variables of loading and/or of adding different ECM components to the gels could be the cause of the release over time.

Much future work could be done based off of this last experiment. First, the LP should be proven to bind HA to PEG, by an ELISA. If the peptide binds then it should be used for further studies. Conversely, if it does not bind the HA to PEG, then another peptide needs to be synthesized that will bind to both HA and PEG. Once a peptide has been sequenced, release studies can then be conducted to study various amount of HA from gels at varying cross-linking densities. Dynamic loading under different frequencies such as our studies of ChS could also be conducted with HA. The release of other ECM components could be studied, instead of just HA and ChS. Similarly, like our ChS loading studies, this study could be drawn out for a longer amount of time to see if there is any change in the rate of release of these or other ECM components.

Conclusion

This summer, the effect of cross-linking density and mechanical loading on the release of ECM components, specifically chondroitin sulfate (ChS) and hyaluronic acid (HA), from PEG hydrogels was studied. It was observed that cross-linking density significantly affects the release of ECM components from the hydrogel where increased ρ_c results in lower release rates.

The frequency of mechanical loading also has profound effects on the release of ECM components, specifically when water content and mesh size is decreased. Future studies will focus on isolating the confounding mechanisms that result in both increases and decreases in release, such as the ones seen under 3 and 1 Hz in the 20% gels.

The addition of the link peptide to the gels appears to interact with both ChS and HA, but the extent of the interaction is still unknown. In some cases, the addition of LP caused a slight decrease in the release of ChS (higher ρ_c) and HA (lower ρ_c) under loading, yet opposite effects were also observed. Further studies will be performed to characterize the specific binding kinetics of these molecules to the peptide.

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Student Perspectives

Karen Horovitz

Mulungu

For most, a painful headache will be followed with an over the counter analgesic pill. However, for many years in Brazil, inhabitants in the Amazonian rainforest have utilized the power of homeopathic medicine. They took advantage of trees, shrubs, and flowers that surrounded them and found practical uses for them. One of the more commonly used plant families, the Erythrina Genus, contains over 100 species of trees and shrubs[6]. This genus includes not only the national flower of Argentina, or the E. Crista-galli, but also the Mulungu and Velutina trees which have exhibited many health benefits to humans.

The Erythrina Genus species contain flavonoids, triterpenes, and alkaloids, which are known to produce treatments for a range of medical problems. Mulungu and Velutina, trees of the Erythrina Genus, have been studied and proven to provide anxiolytic, antibacterial, and antidepressant effects. In a 2007 study published by Flausino OA Jr. et al at University of Sao Paulo, erythrian alkaloids are shown to cause anxiolytic effects in animal models of anxiety[1,2]. In another study, published in 2006 by Ribeiro et al, both Mulungu and Velutina show anxiolytic and antidepressant effects in animal models of anxiety and depression[5]. Another study, also published in 2006, by de Lima MR et al, shows that Mulungu as well as some other Brazilian medicinal plants show antibacterial activities[1]. Specifically, the plants showed antibacterial activity in the presence of *Staph* and *E. Coli*. One other study published by Khaotrek et al in 2008 reveals that flavonoids in Erythrina fusca show antimalarial activity[4]. All these studies, among many others, emphasize on the health benefits that alkaloids and flavanoids in Erythrina plants produce.

Homeopathic medicine has been controversial because many believe that it is not as effective as conventional medicines. However, it is important to remember that modern chemicals have natural origins. Aspirin is derived from salicylic acid, which was once used in the form of salicylate-rich willow bark. This can explain how there may be many other plants that are beneficial, yet we have not figured out a way to produce the chemicals they hold in a laboratory. Next time, think again before you reach for an aspirin. Homeopathic medicine shows promising results, and this is reflected by its use in history.

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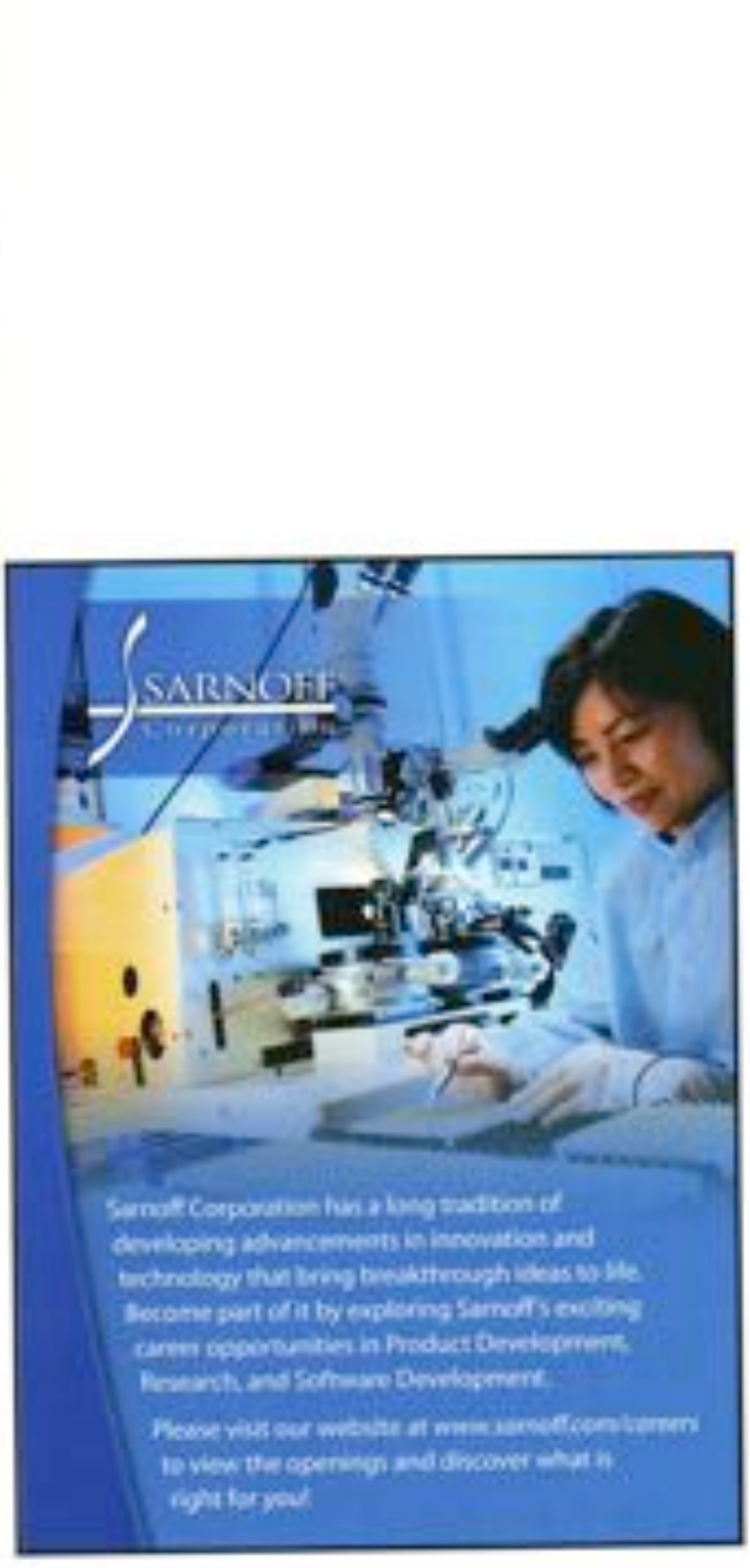

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Synthesis and Characterization of $Fe_3Cl_6(NMP)_8$

Ferdous Zannat, 2009
Adviser: Dr. Patrick Holland
Department of Chemistry

Cross-coupling reactions are dominated by the use of nickel and palladium catalysts.[1] In 1971, Kochi et al. showed that iron salts can be used for a similar purpose.[2] Iron catalysts were cheaper and less toxic than the palladium and nickel catalysts. Kochi et al. found that alkenyl halides undergo stereospecific cross-coupling with alkyl Grignard reagents in the presence of catalytic amounts of FeX_3. However, alkenyl halides had to be used in excess.[3] This limitation was overcome by Cahiez et al. who showed that using NMP as a coolvent significantly increases the yield of the reaction.[4] For instance, when 1-bromoprop-1-ene as reacted with octylmagnesium chloride in the presence of 3% $Fe(acac)_3$ it gave only 40% of 2-undecene (Scheme 1). However, when NMP was added as a coolvent, the yield was 87%. Cahiez et al. proposed that NMP improved the yield by stabilizing the iron containing catalytic intermediate, by limiting the decomposition process. However, to our knowledge, no iron-NMP complexes of any kind have been isolated. Furthermore, no NMP coordinated transition metal complexes have been isolated. The only examples of coordinated NMP are with the lanthanoid metals such as La^{3+}, Ce^{3+}, Pr^{3+}, and Gd^{3+}.[5,6] Thus, in this study we aim to investigate how NMP coordinates to a transition metal, particularly Fe(II). A NMP -like compound, such as N,N'- ethylenebis(pyrrolidin-2-one) (ebpyrr) has been shown to form four and/or six coordinated complexes with transition metals such as Co(II), Zn(II) and Cd(II) (Figure 1).[7] Furthermore, an octahedral symmetry was observed when the metal, in the study Zn(II), was coordinated to only one type of ligand, ebpyrr. Thus, our hypothesis is that Fe(II) will also form an octahedral complex with coordinated NMP.

Results and Discussion

NMP has been shown to increase the yield when used as a coolvent with THF in iron catalyzed cross coupling reactions.[8] Therefore, it is of interest to examine any interaction between NMP and iron. A reaction between an iron salt, $FeCl_2(THF)_{1.5}$ and ten equivalents of NMP in THF at 25°C for two hours resulted in a clear solution, which was kept at -44°C overnight to yield light purple crystals (1). The yield is 62% and the elemental analysis is consistent with a stoichiometry $FeCl_2(NMP)_{2.5}$. Product 1 was characterized by the X-ray crystallography, ^1H NMR, IR, UV-Vis spectroscopy, and elementalanalysis. The X-ray crystal structure (Figure 2) shows that the product is $Fe_3Cl_6(NMP)_8$ with a six-coordinated octahedral $Fe(NMP)_6^{2+}$ cation and two tetrahedral $FeCl_3(NMP)^-$ anions that are related by a crystallographic inversion center. Selected bond distances and angles for $Fe_3Cl_6(NMP)_8$ are shown in Table 1.

Scheme 1. Cross-coupling of octylmagnesium and 1-bromoprop-1-ene.[4]

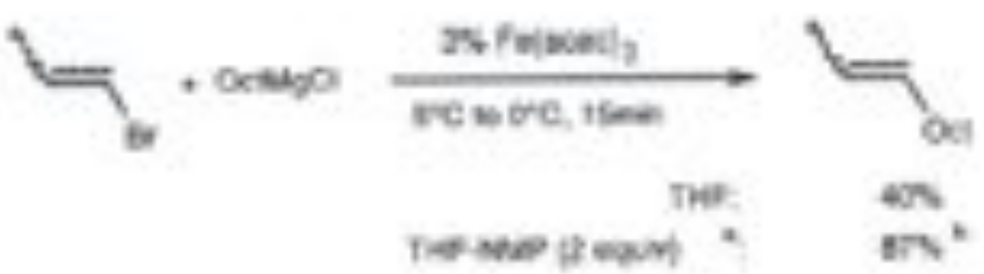
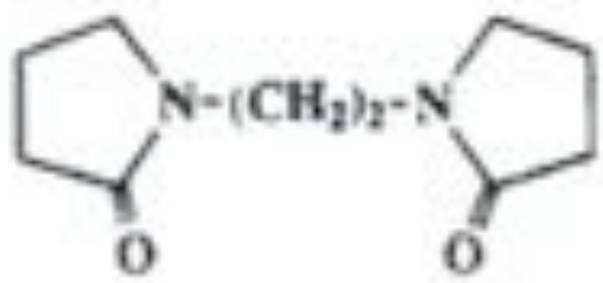


Figure 1. Structure of N,N'-ethylenebis(pyrrolidin-2-one) (ebpyrr).[7]



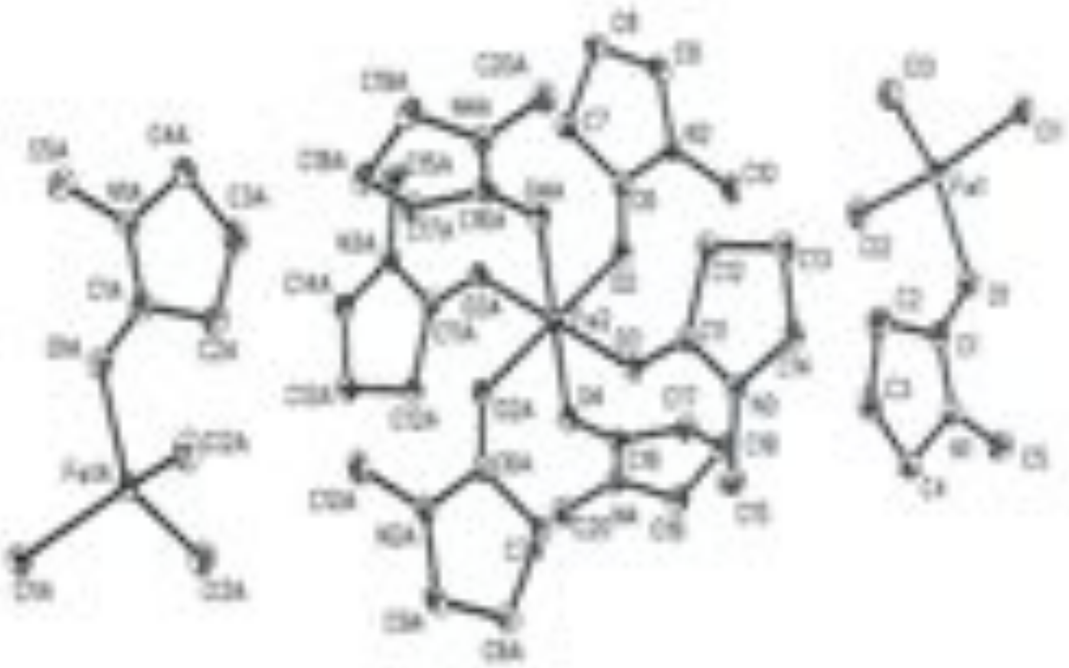


Figure 2. Molecular structure of $Fe_2Cl_6(NMP)_2$

Table 1. Selected bond lengths (Å), angle (°), and esd's for $Fe_2Cl_6(NMP)_2$

1. Iron-Oxygen Distances and Angles in Six Coordinated Iron Cation

Fe(2)-O(2)	2.1290(8)
Fe(2)-O(3)	2.0998(8)
Fe(2)-O(4)	2.1655(8)
Mean	2.13(3)

Fe(2)-O(2)-C(6)	132.02(8)	O(2)-Fe(2)-O(3)	90.15(3)
Fe(2)-O(3)-C(11)	136.51(8)	O(3)-Fe(2)-O(4)	89.86(3)
Fe(2)-O(4)-C(16)	129.51(8)	O(2)-Fe(2)-O(2A)	180.00(5)
Mean	133(4)		

2. Iron-Oxygen and Iron-Chloride Distances and Angles in Four Coordinated Iron Anion

Fe(1)-O(1)	2.0853(9)
Fe(1)-Cl(1)	2.2858(4)
Fe(1)-Cl(2)	2.2959(4)
Fe(1)-Cl(3)	2.2921(4)
Fe(1)-Cl(3)	2.291(5)

Cl(1)-Fe(1)-O(1)	102.57(3)
Cl(1)-Fe(1)-Cl(3)	109.131(15)
Cl(2)-Fe(1)-Cl(3)	108.635(15)
Cl(2)-Fe(1)-O(1)	97.36(3)
Fe(1)-O(1)-C(1)	124.46(9)
O(1)-Fe(1)-Cl(3)	118.68(3)

3. Carbon-Oxygen, Carbon-Carbon, Carbon-Nitrogen Distances in Six Coordinated Iron Cation

C-O	Distance	C-C	Distance
O(2)-C(6)	1.2499(14)	C(6)-C(7)	1.5005(16)
O(3)-C(11)	1.2499(14)	C(7)-C(8)	1.5286(16)
O(4)-C(16)	1.2468(14)	C(8)-C(9)	1.5350(18)
Mean	1.249(2)	Mean	1.52(2)

C-N	Distance	N-Meth	Distance
C(6)-N(2)	1.3361(15)	N(2)-C(10_	1.4519(16)
C(11)-N(3)	1.3298(15)	N(3)-C(15)	1.4577(15)
C(16)-N(4)	1.3351(15)	N(4)-C(20)	1.4454(16)
Mean	1.333(3)	Mean	1.452(6)

4. Carbon-Oxygen, Carbon-Carbon, Carbon-Nitrogen Distances in Four Coordinated Iron Cation

C-O	Distance	C-C	Distance
O(1)-C(1)	1.2257(16)	C(1)-C(2)	1.5008(19)
		C(2)-C(3)	1.5295(19)
		C(3)-C(4)	1.538(2)
		Mean	1.52(2)

C-N	Distance	N-Meth	Distance
C(1)-N(1)	1.3242(16)	N(1)-C(5)	1.4529(17)

Table 2. Selected bond lengths (Å) and angles (°) for tetrahedral $C_{12}H_{16}C_{o}CpN_2O_2$ [7]

Co-Cl(1)	2.222(3)	Co-Cl(2)	2.224(3)
Co-O(1)	1.980(5)	Co-O(12)	1.984(5)
Cl(1)-Co-Cl(2)	119.1(1)	Cl(3)-Co-O(1)	103.9(2)
Cl(2)-Co-O(1)	114.2(2)	Cl(1)-Co-O(12)	109.6(2)
Cl(2)-Co-O(12)	10.5(2)	O(3)-Co-O(12)	103.6(2)
Co-O-C(1)	133.0(5)	Co-O(12)-C(12)	126.8(5)

Table 3. Selected bond lengths (Å) and angles (°) for tetrahedral $C_{12}H_{16}I_2N_2O_2Zn$ [7]

Zn-I(1)	2.553(1)	Zn-I(2)	2.570(1)
Zn-O(1)	2.000(4)	Zn-O(12)	1.975(5)
I(1)-Zn-I(2)	121.3(1)	I(1)-Zn-O(1)	105.8(1)
I(2)-Zn-O(1)	107.8(1)	I(1)-Zn-O(12)	114.6(1)
I(2)-Zn-O(12)	106.3(2)	O(1)-Zn-O(12)	98.8(2)
Zn-O(1)-C(1)	126.2(4)	Zn-O(12)-C(12)	137.4(5)

Doyle et al. characterized tetrahedral and octahedral complexes of ebpyr coordinated to Co(II) and Zn(II) by X-ray crystallography. [7] The crystal structures of the tetrahedral and the octahedral cobalt and zinc complexes are in shown in Figures 3 and 4, respectively. Selected bond lengths (Å) and angles (°) for the complexes are shown in Tables 2-5. [7]

Table 4. Selected bond lengths (Å) and angles (°) for octahedral $C_{42}H_{70}CoN_2O_8$.[7]

Co-O(12)	2.062(9)	Co-O(1)	170.8(7)
Co-O(31)	2.080(12)	Co-N(42)	89.3(5)
Co-O(22)	2.192(11)	Co-O(21)	99.6(6)
O(12)-Co-O(1)	92.3(4)	O(12)-Co-O(31)	170.8(7)
O(1)-Co-O(31)	83.6(5)	O(12)-Co-N(42)	89.3(5)
O(1)-Co-N(42)	100.9(6)	O(31)-Co-N(41)	99.6(6)
O(12)-Co-O(22)	81.6(4)	O(1)-Co-O(22)	132.7(7)
O(31)-Co-O(22)	98.3(4)	N(42)-Co-O(22)	105.5(7)
O(12)-Co-O(21)	85.3(4)	O(1)-Co-O(21)	94.3(5)
O(31)-Co-O(21)	86.8(5)	N(42)-Co-O(21_	164.0(5)
O(22)-Co-O(21)	58.8(6)	C(1)-)(1)-C0	128.3(13)
C(12)-O(12)-Co	140.6(8)	N(20)-O(22)-Co	92.8(12)
N(20)-O(21)-Co	91.2(14)	N(30)0O(31)-Co	120.5(14)
C(41)-N(42)-Co	172(2)		

Although the metal-oxygen bonds have different lengths in the tetrahedral complexes of Co(II), Zn(II) and Fe(II), a pattern is observed. The bond lengths increase going from zinc to cobalt to iron (Table 3, 2, 1B). The pattern is due to a larger ionic radius when going from zinc to cobalt to iron. The metal-halogen bonds are also different in the three complexes. The iron- chlorine bond is longer than the cobalt-chlorine bond also because of the larger ionic radius of iron compared to cobalt. The zinc-iodine bond is larger than both because iodine has a larger atomic radius compared to chlorine. The metal-oxygen bond lengths are also different in the octahedral complexes of Fe(II), Co(II), and Zn(II) (Table 1A, 4, 5). The difference as mentioned above is due to an increased ionic radius when going from zinc to iron. However, the metal-oxygen-carbon and oxygen- metal-oxygen angles in zinc and iron complexes are not different. Thus, NMP coordinated

Table 5. Selected bond lengths (Å) and angles (°) for octahedral $C_{36}H_{54}Cl_2N_2O_{10}Zn$.[7]

Zn-O(12)	2.107(2)	Zn-O(22)	2.104(2)
Zn-O(32)	2.112(2)		
O(12)-Zn-O(22)	88.46(9)	O(12)-Zn-O(22a)	91.54(9)
O(12)-Zn-O(32)	90.34(10)	O(12)-Zn-N(32a)	89.66(10)
O(22)-Zn-O(32)	90.58(10)	O(31)-Zn-O(32a)	89.42(10)
O(12)-Zn-O(12a)	180.0	O(1)-Zn-O(22a)	180.0
O(32)-Zn-O(32a)	180.0	C(12)-O(12)-Zn	135.9(2)
C(22)-O(22)-Zn	131.2(2)	C(32)-O(32)-Zn	131.8(2)

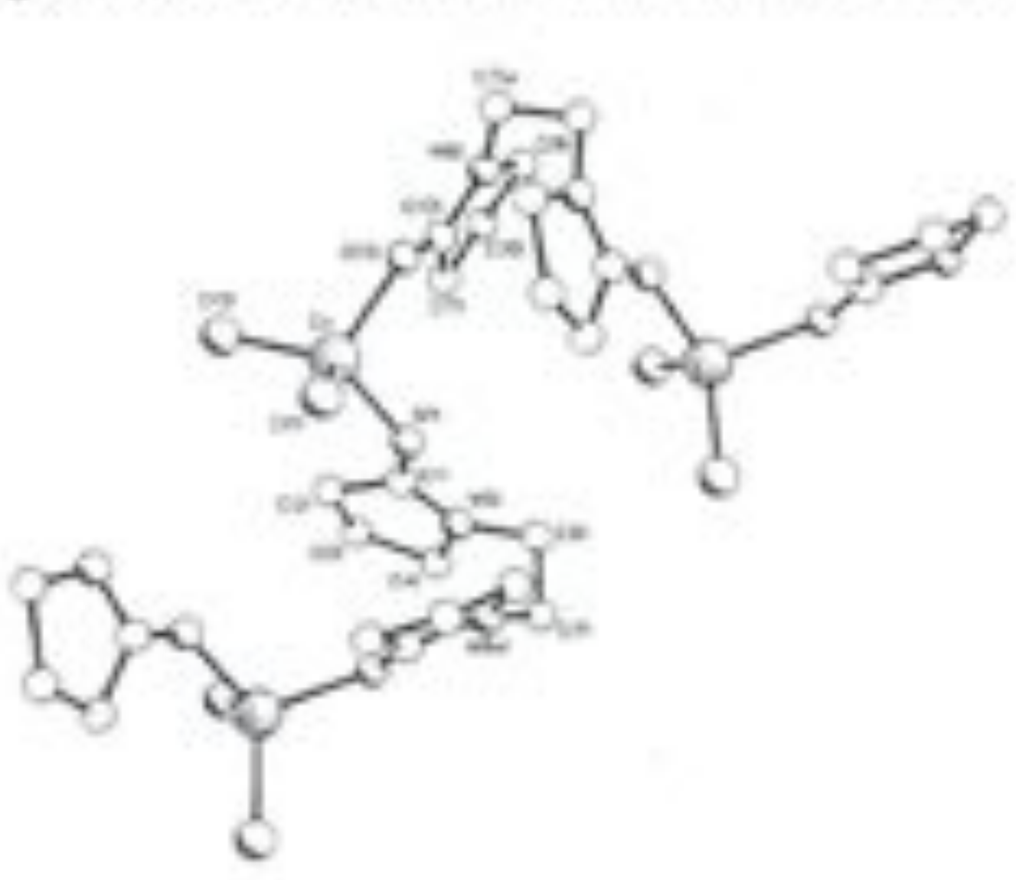
iron complex is structurally similar to ebpyrr coordinated zinc complex. Both complexes are octahedral. Moreover, the zinc compound formed a tetrahedral geometry when coordinated to ebpyrr and halogens (Figure 3). Similarly, one of the iron centers also has a tetrahedral geometry when coordinated to NMP and halogens (Figure 2). The cobalt complex also formed a tetrahedral geometry when coordinated to halogen (Figure 3).

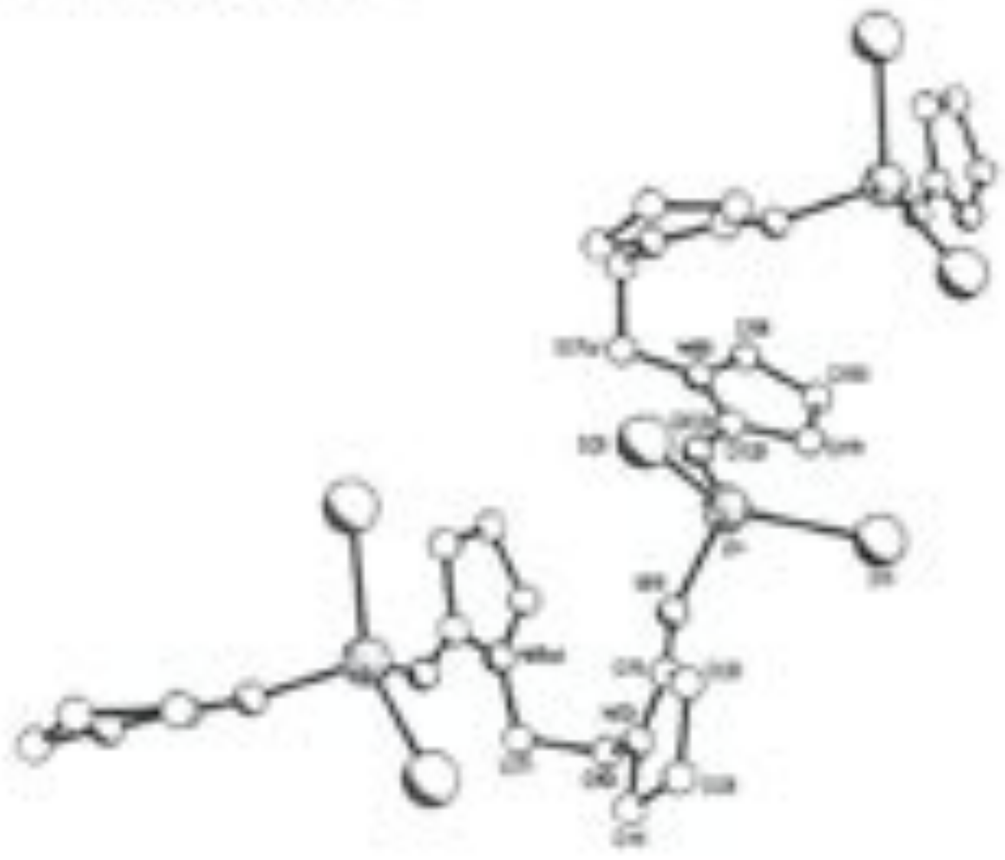
^{1}H NMR spectra for $Fe_2Cl_6(NMP)_2$ in $THF\text{-}d_8$ showed peaks corresponding to coordinated NMP (Table 6). The compound is paramagnetic since a peak at δ -1.38 ppm is observed. The X- ray crystal structure showed two different environments for NMP. However, ^{1}H NMR spectra shows both types of NMP to be equivalent. This suggests a rapid exchange of NMP between the two positions. Also, the carbon-carbon, carbon-oxygen, and carbon-nitrogen bond lengths are not different (Table 1-3, 1-4). Thus, the two types of NMP have chemically equivalent bonds.

Table 6. ^{1}H NMR peaks in $Fe_2Cl_6(NMP)_2$

Peaks (ppm)	**Integration**	**Assignments**
9.32	2 H	O=C-CH_2
3.01	3 H	N-CH_3
1.86	2 H	N-CH_2-C or OCH_2-C
-1.38	2 H	N-CH_2-C or C-CH_2-C

Figure 3. Molecular structure of tetrahedral complex $C_{36}H_{58}Cl_2Cl_2N_2O_2$ and $C_{36}H_{54}I_2N_2O_2Zn$.[7]





UV-Vis spectra showed a peak at 360 nm (ε = 21 $M^{-1}cm^{-1}$). In the following discussion, we assume that this is a d-d transition of Fe(II), which is a d^6 ion where B is 1058 cm^{-1} for the free ion. A high spin d^6 ion (based on NMR peaks) has four unpaired electrons, which corresponds to a calculated magnetic moment of 4.9 B.M. In the future, we will measure the magnetic moments. The E/B is 26 and the Δ_o/B is 31 for the peak at 360 nm. Thus, Δ_o is calculated to be 32,800 cm^{-1}. IR spectra carried out in KBr pellet showed a strong C=O band at 1650 cm^{-1}. The C=O stretching frequency is consistent with other literature stretching frequencies for NMP coordinated to main group metals such as Ca(II), Mg(II), Zn(II) and more. [9] The IR spectra also showed a weak C-H band at 2930 cm^{-1}.

Conclusions

The reaction of $FeCl_2(THF)_{1.5}$ with NMP formed $Fe_2Cl_4(NMP)_4$. The compound was characterized by the X-ray crystallography, ^1H NMR, IR, UV-Vis spectroscopy and elemental analysis. The X-ray structure showed a six coordinated iron center. Thus, the hypothesis was correct; NMP formed an octahedral complex with iron. However, the unit cell also had four coordinated iron centers with coordinated chorine and NMP ligands. Literature showed that four coordinated metal centers are typically observed when halogen ligands are present. Thus, the tetrahedral iron in $Fe_2Cl_4(NMP)_4$ followed the same pattern. In future studies, whether $Fe_2Cl_4(NMP)_4$ is a better catalyst than $FeCl_2(THF)_{1.5}$ will be determined by carrying out cross coupling reactions, and the coordination of NMP to other iron salts such as $Fe(acac)_3$ and $Fe(acac)_2$ will be investigated.

Experimental

General Considerations. All manipulations were performed in a glove box filled with N_2 or Ar. Glassware was dried at 150 °C overnight. NMR data were recorded on a Bruker Avance 400 spectrometer (400 MHz). Infrared spectra (1000-4000 cm^{-1}) were recorded on KBr pellet samples in a Shimadzu FTIR spectrophotometer (FTIR-8400S) using 16 scans at 2 cm^{-1} resolution. UV-Vis spectra were measured on a Cary 50 spectrophotometer using screw-cap cuvettes. Elemental analyses were determined by Desert Analytics, Tucson, AZ.

Synthesis of $Fe_2Cl_4(NMP)_4$. In a glove box filled with N_2, $FeCl_2(THF)_{1.5}$ (250 mg,1.06 mmol), THF (20 mL), and a stir bar were added to an oven dried scintillation vial. NMP (1.03 mL, 10.6 mmol) was added slowly using a 1 mL syringe. The solution was stirred for 2 h and filtered through a pipette filter using Celite into a scintillation vial. The solution was kept at -44 °C overnight to yield light purple crystals which was then pumped down (62 %). ^1H NMR (400 MHz, THF-d8) : δ 9.73 (2H, O=CCH), 5.01 (3H, N-CH), 1.86 (2H, C-CH-C or N-CH), -1.38 (2H, C-CH-C or N-CH) ppm. UV-Vis (THF): 360 (ε = 21 $M^{-1}cm^{-1}$). IR (KBr pellet): 1650 (s) (C=O), 2930 (w) (C-H) cm^{-1}. Elem. Anal. Calcd. for $Fe_2Cl_4(NMP)_4$: C, 40.95; H, 6.18; N, 9.55. Found: C, 41.04; H, 6.25; N, 9.26.

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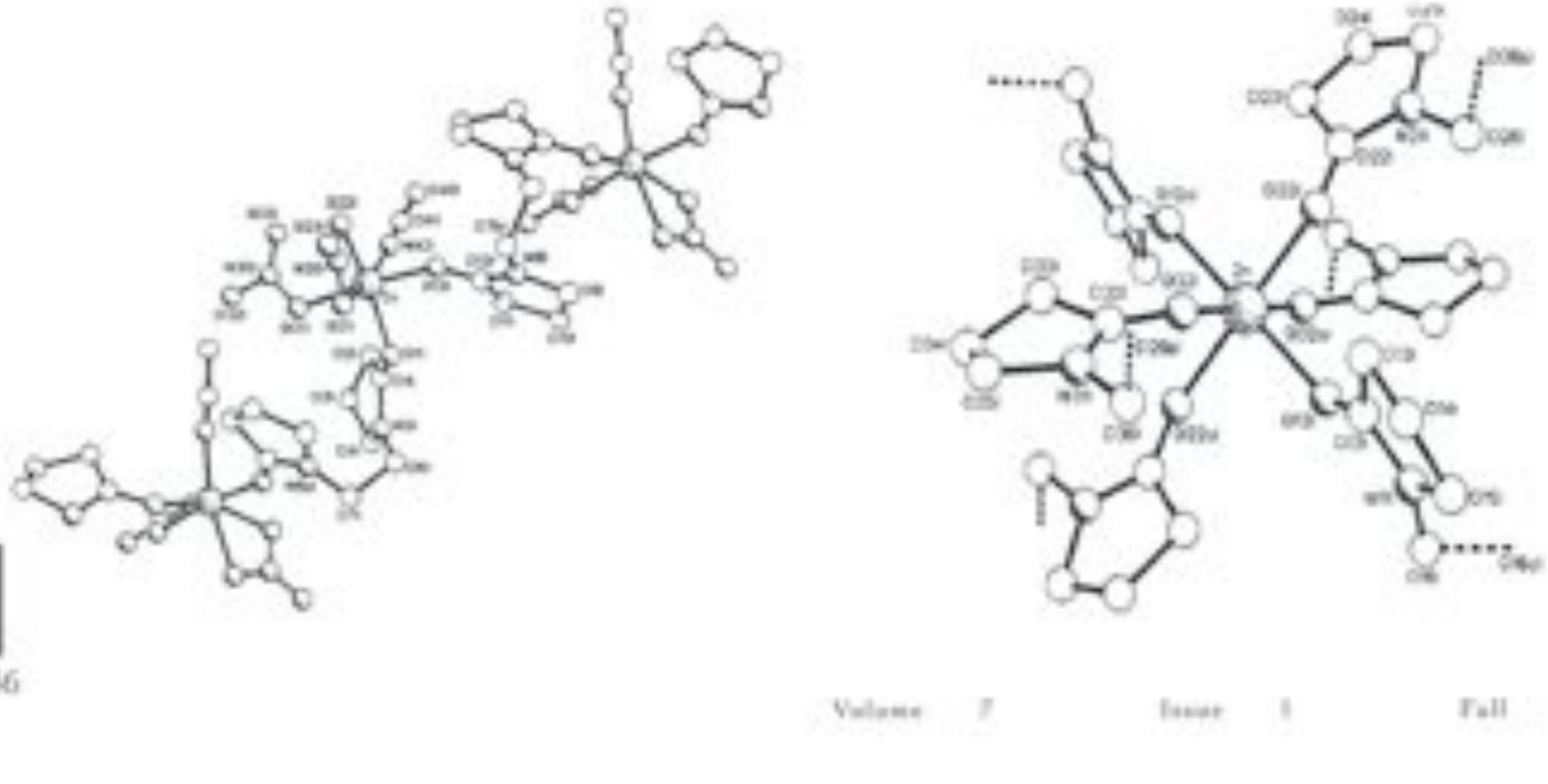
Figure 4. Molecular structure of octahedral complex $C_{12}H_{18}CoN_2O_4$ and $C_8H_{14}Cl_2N_2O_{10}Zn$.[7]



Student Interview

Cody R. DeHaan

JUR: What kind of research are you involved in?

For the past five semesters, I've been doing motivation and self-determination research with Professor Richard Ryan and Netta Weinstein in the Psychology department. We do research in motivation in the domain of Self-Determination Theory, such as intrinsic and extrinsic motivation, as well as experiences of being in nature, reactions to stress, mindfulness and helping behaviors.

JUR: How did you become interested in this topic?

Actually, I wasn't initially interested in research, but first semester freshman year I took a course with Professor Ryan. Toward the end of it, he announced to the class that he was looking for research assistants. I applied thinking it might be interesting, and I had an interview with Netta and she offered me a position. I actually spent my first semester running stress studies. I became interested in it after that. I wasn't initially interested but I really grew to like it.

JUR: Is research the experience that made you decide that Psychology was the right major for you?

It definitely helped. I was torn between Computer Science and Psychology when I first started out but as I got further involved in psychology research and course material, I realized that I was interested in pursuing Psychology.

JUR: Do you think it has helped you see how the research process works?

Definitely. I had some idea about how it worked beforehand, but I didn't understand how much work is involved on so many levels. But now when I read research, or read about research in textbooks, I have the knowledge to understand the conclusions, the process behind them, and read with a deeper understanding.

JUR: Why is research important to you?

Research is important to me because it's something I plan to do in the future. It's also the way in Psychology that we discover – or try to discover – how different processes actually work. You can sit down and think all day about, "how does this work," "what motivates me to do this," "what reasons do I have to do this" "why do I think this way about things," "why do other people think about things this way"? Research gives you a way to take these and to prove them, to make them concrete, to test them, to actually show which ideas and theories actually hold true under certain circumstances.

JUR: Do you see that as the goal of psychological experiments?

That's one of the goals. Definitely applying that research and helping people is one, but some of it is just fun and interesting to see – all these different things that people do and some of the reasons that might be behind them; basically, searching for what makes people tick. One of the best things is that it can be applied to people and used to make people's lives better, whether they have a difficulty they're suffering from, or whether they're functioning in a healthy way and want to improve themselves.

JUR: What do you plan to do with this research in the future? Do you have any specific plans?

Well, I plan on going to graduate school after finishing undergrad. I'm not exactly sure what specific path I'd like to go down yet, but I know I'll end up doing more research because I really enjoy it. I guess I'm just going to follow my interests and see where it takes me.

JUR: Is there anything you want to add?

I just think that people who have the ability to do research should pursue it. You know, you can look around and find an opportunity that is 2 credits, so it's not a huge commitment. If you have the opportunity, it's definitely something to check out – it hugely influenced my career path.

JUR: Any other advice?

Any worries you have are actually paid off by whether or not you liked the experience. You can have it be a really small time commitment, even. Most of the faculty are very open to students and to go into a professor's office hours to talk to them to see if they have any research opportunities and might be willing to take you on. Just try to be open to new opportunities and be open to asking questions.

JUR: Are you doing your own research now?

I'm actually conducting my own research, in fact. We're finishing up one study this semester. I'm also working on a paper with the graduate student I'm working with, Netta Weinstein, and we're hoping to get that out soon. We're progressing down some relatively unexplored and interesting paths, so it'll be interesting to see how those projects turn out.

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Potential of Triploid English Ivy (Hedera spp., Araliaceae) as a Less Invasive Substitute

Emily Reiss, 2008
Advisers: Udo Fehn, Ph.D., Robert Minckley, Ph.D., Justin Ramsey, Ph.D.
Department of Earth and Environmental Sciences

English Ivy (*Hedera*) is a genus of evergreen woody vines native to Eurasia, but introduced to North America in the early 1700's (Rose, 1996). It is a popular horticultural choice across the United Sates and is planted for aesthetic as well as functional reasons, such as erosion control. However, ivy is one of many invasive species in the United Sates that poses a real threat to native species and is a serious problem for communities throughout the United States especially in the Pacific Northwest and Mid-Atlantic states. Through seed transportation by birds, fertile, fruiting ivy in a cultivated garden can spread long distances. When ivy is introduced in an isolated wood lot, it can wreak havoc in the forest ecosystem by crowding and shading native species.

Invasive species are any non-native organisms that out-compete native species, change community structure, and alter ecosystem processes (Ramsey, pers. comm.). Not all non-native species are invasive, but those that are can be dangerous and often cause costly damage. In the United States almost $1.2 billion is spent annually on management of invasive species (Ramsey, pers. comm.). The taxonomy of English Ivy is complicated, but it is generally believed that there are fifteen species within the genus world-wide. (Rose, 1996). The invasive populations in North America are dominated by two alien species, *Hedera helix* (2x) on the eastern coast and *H. hibernica* (3x) on the western one (Green, Ramsey and Ramsey, in prep.).

Ivy's invasiveness is directly related to its life cycle. In the juvenile stage ivy forms a dense ground cover. At this point it can cover the forest floor preventing seed germination and shading out other understory herbs (Biggerstaff and Beck, 2007). When ivy encounters a vertical surface, it begins to climb, putting out adventitious roots to assist in its ascent. In a forest, ivy will climb trees and transition to an adult form. Dense ivy growth in the canopy further diminishes the sunlight reaching the forest floor, and the weight of the vine can ultimately kill a host tree. Additionally the adult ivy will begin to flower and fruit.

Recent data indicate that seed distribution by frugivore (fruit-eating) birds is the primary mode of new ivy patches in natural forest habitats (Ramsey, 2005). A typical scenario is as follows: an ivy plant in a residential area fruits, attracting birds that consume the berries and fly to a forested area; at this point the birds defecate or regurgitate the seeds and ivy plants colonize a previously "uninfected" wood lot. Consumed ivy seeds have a germination rate of almost 100% and start to germinate within a couple of weeks (Georgeau, 1992). Ivy is unusual as an invasive species in that it does not need a previously disturbed area in order to invade, making it significantly more harmful (Reichard and White, 2001). These facts, coupled with vigorous vegetative growth, mean that ivy can drastically change the landscape of a natural area in a short amount of time, even when parental plants are several kilometers away. To avoid the problems posed by invasive ivy, many communities have attempted to keep populations under control. The most successful method is manual removal of individual plants from natural areas. This method immediately eliminates ivy from the area with little chance of regrowth, but as long as there are fertile ivy plants in the vicinity, control will be fleeting.

Given the impact of fertile ivy plants, a sterile strain could minimize the invasiveness of ivy. Polyploidy is a naturally occurring phenomenon that can render individuals sterile. Polyploid individuals have more than two sets of chromosomes (>2x), and can be found in a variety of organisms, including amphibians fish and more than half of the flowering plants (Ramsey and Schemske, 1998). When an organism reproduces, it undergoes a meiotic process in which its genetic material (chromosomes) replicate, align, and pull apart to produce four new cells (gametes) each with half the original number of chromosomes. Certain polyploids, triploids (3x), have only three sets of chromosomes, which makes meiosis nearly impossible because the three sets cannot align properly to produce new cells (offspring). Consequently, triploids are often sterile (Ramsey and Schemske 1998). Triploids can form spontaneously, or as a hybrid of a diploid (2x) and tetraploid (4x). In the Old World, populations of *Hedera* (2x and 4x) are geographically separated which minimizes the opportunity for hybrid triploid formation. In North America, however, sympatric populations of 2x and 4x species of *Hedera* increase the possibility of triploid hybrids.

In horticultural settings, triploids are desirable for their hybrid vigor, a phenomenon in which individuals exhibit more vigorous growth because reproductive energy can be redirected towards vegetative growth (Ramsey and Schemske 1998, 2002). Wildlife biologists have long used triploid trout for this reason. By expos-

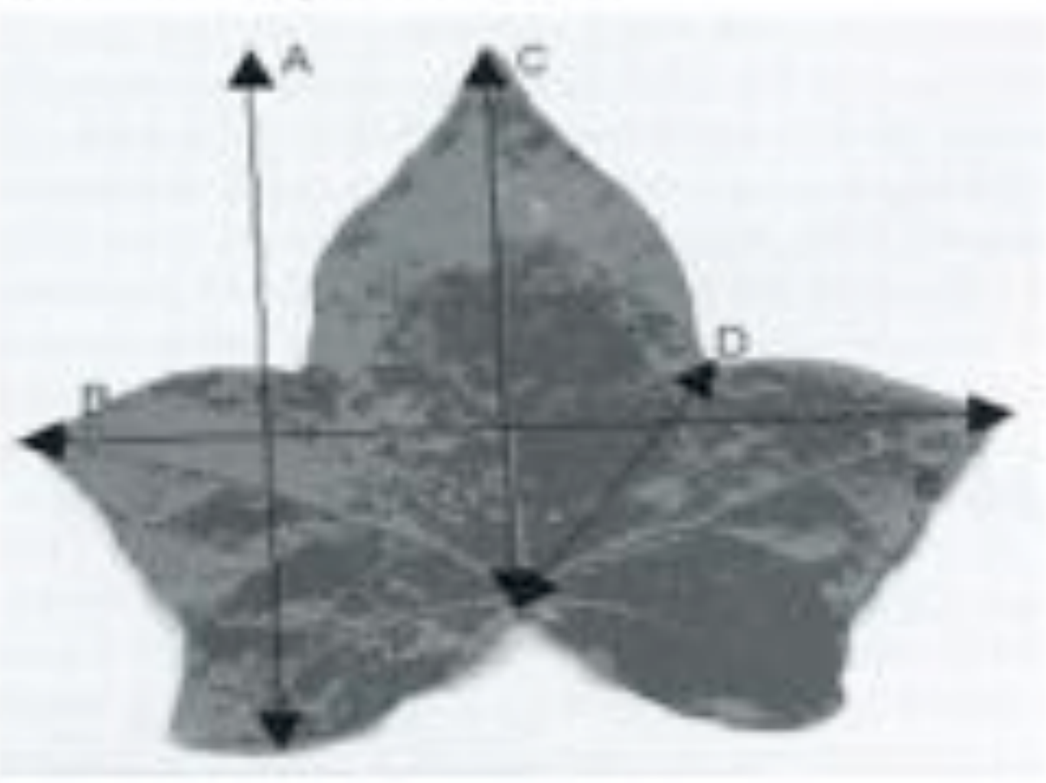
ing eggs to heat shortly after fertilization it is possible to create large, sterile rainbow trout which can be stocked along with native populations without any risk of interbreeding (Thorgaard, Jazwin, and Stier, 1981). Similarly, the discovery of triploid individuals in the genus *Populus* provided an additional and more rapid path to the development of longer-lived and faster-growing individuals for cultivation (Bradshaw and Stettler, 1993).

Through extensive field sampling in the Seattle area, Tara Ramsey (Research Associate, Department of Biology, University of Rochester) encountered several triploid ivy individuals. Subsequent collections by Adam Green (Graduate Student, University of Rochester) have revealed additional triploid plants on both the East and West coasts (Green, pers. comm.) While triploids can still spread vegetatively, without a fruit set they would not be a horticultural source for long-distance dispersal like traditional ivy varieties.

The main barrier to the wide-spread use of triploid ivy is integration into the horticultural market, and acceptance by wholesale distributors and individual consumers. Ivy is desirable for its morphological variety and versatility as a ground cover. Additionally, many consider ivy an effective means of erosion control and it is planted by landscapers and highway maintenance crews. Efforts to remove ivy altogether from the horticultural market have been met with resistance by nursery owners and homeowners. Triploid ivy could serve as a compromise, offering a plant with a limited capacity for invasion of natural areas coupled with the desired traits of traditional ivy, both in appearance and growth patterns.

To provide insight into the potential for horticultural acceptance of triploid ivy, three co-occurring cytotypes (triploid, diploid and tetraploid) found in a Seattle State park were grown in a controlled environment. Horticulturally important traits were measured and analyzed for statistically significant differences that would promote or hinder the integration of sterile triploids into the horticultural market. The data suggest that triploid ivy shares many traits with currently marketed cytotypes of ivy and may exhibit additional advantageous traits. In particular, triploid ivy leaves are similarly lobed compared to tetraploids, but in fact triploids have more leaves per plant, which could be beneficial in erosion control situations. Additionally, triploids show the greatest shoot mass. Based on these and the following results, I speculate that triploid ivy could be successfully introduced into the horticultural market. Ideally this introduction would coincide with the phasing out of fertile and more invasive forms of ivy.

Figure 1. Leaf Measurements
A = Maximum Length, B = Maximum Width, C = Base to Tip, D = Base to Sinus, E = Base to Lobe



Methods

The study individuals were selected from collections made by Tara Ramsey from St. Edwards State Park (Bothell, Washington). The use of specimens from the same site minimizes the differences in morphology and fitness for each cytotype, which may be affected by regional or environmental effects. Additionally the sympatric presence of *H. hibernica* and *H. helix* at the sample site increases the probability of *in situ* origin of the triploids found in the park.

Plant propagation All plant propagation was carried out in the departmental plant growth room at the University of Rochester in Hutchinson Hall (221A). The 220 sq. ft. space has multi-tiered metal shelving units with four grow lights suspended on each level. The room also has watering and fertilizing facilities. Specimen selection was based on the plant material available for cuttings. With these criteria, three individuals (genotypes) were selected from each of the three cytotype categories (2x, 3x, 4x) and five cuttings were made from each individual, for a total of 45 cuttings. A potting soil mix was watered thoroughly, and cuttings were planted in labeled 7.5 cm round pots. Every attempt was made to make cuttings uniform but the plants varied in stem length from 15 to 45 cm. Eight pots were arranged in a standard 25x50 cm flats, covered with a clear plastic dome to maintain humidity, and misted daily. After three weeks, covers were removed, and plants remained in the flat with regular misting and watering. At four weeks the pots were removed from the flats and randomized on growth room shelves. Plants were subsequently randomized at six and eight weeks. Fertilizer (1 tablespoon of 20-20-20 fertilizer mix per three gallons) was administered equally to all plants during weeks five, seven and nine.

Plant measurements Each individual was measured for stem girth, and the number and color of leaves a week before harvest. Color value was determined based on a standard six-value color chart. Plants were then harvested by extracting plants from pots and removing the soil, achieved by dipping the root mass in water. The plants were air dried and then arranged in a herbarium press and left to dry for 10 days. At this point, each plant was measured for the number and length of stems, petiole length, and internodal number and distance. Additionally five measurements were made on each leaf: base to tip, maximum length, maximum width, base to sinus, and base to lobe. Finally, roots were separated from the stem and both were weighed separately.

The measurements were selected for either fitness or morphological significance. Total stem length along with stem number and girth, and shoot mass provide an indication of the vigorousness of growth. Root mass is necessary to calculate the root to shoot ratio which can be significant in terms of erosion control. Inter-nodal distance and number can provide additional information as to the density of the leaf mass, important for both fitness and morphology. The five leaf measurements allow for ratio calculations such as sinus:lobe, or lobedness, a typical morphological characteristic. These measurements also inform leaf mass and size which can affect water use. Leaf number, color, and petiole length are more exclusively morphologically important. (Figure 1)

Flow Cytometry To confirm the ploidy level of the six purported

triploid individuals used in the study, 5 g of fresh leaf tissue was placed in a petri dish with 2 ml of buffer (3.6 g HEPES, 2 ml of a 0.5M solution of EDTA, 6.0 g KCl, 1.2 g NaCl, 102.7 g sucrose, 2 ml Triton X-100, 1 ml ß-mercaptoethanol and 0.1 g spermine in 1.0 L distilled water) and chopped for approximately 1 min by hand with a razor blade. The subsequent mixture was then drawn through a syringe filter (25 mm Millipore Swinnex filter holder, Fisher Scientific Company LLC, Pittsburgh, PA, SX0002500) fitted with 48 µm nylon mesh (Small Parts Inc., Miami Lakes, FL, B-CMN-48) and centrifuged for 1 minute at 10,000 X g. The nuclei pellet was resuspended in 400 µL chopping buffer containing 10 µl of a 5 mg / ml. solution of propidium iodide (PI) and 0.24 µL RNAse A (Qiagen, Valencia, CA). Nuclei from diploid individuals of *Chamerion angustifolium* (Onagraceae), were added to each sample as a control.

Samples were run on a FACS Calibur flow cytometer (Becton-Dickinson, Franklin Lakes, NJ) outfitted with a blue argon laser (488 nm wavelength) in the Cell Sorting facility in the Flow Cytometry and Immunological Analysis Center, University of Rochester, Rochester, NY. Samples were analyzed for relative fluorescence ("FL2-A"), which was summarized as a frequency histogram using CellQuest(TM (v. 5.2.1, Becton-Dickinson). Based on work by Adam Green (Green, pers. comm.) the ploidy levels were calculated based on the relationship between the 2C DNA content of *C. angustifolium* and the 1C DNA of *Hedera* samples.

Data Analysis Statistical analysis was done using the JMP software package (JMP statistical software, SAS Institute, Cary, NC, USA). Traits were analyzed for statistical significance ($P \leq .05$) using ANOVA with post-hocs based on student t-tests. Traits with multiple measurements such as those for leaves were modeled against both cytotype and genotype with genotype specified as a randomly nested factor. For traits with only one measurement, only cytotype was included as a factor in the analysis.

Results

Statistical analysis was done using the JMP software package (JMP statistical software, SAS Institute, Cary, NC, USA). Traits were analyzed for statistical significance ($P \leq .05$) using ANOVA with post-hocs based on student t-tests. Traits with multiple measurements such as those for leaves were modeled against both cytotype and genotype with genotype specified as a randomly nested factor. For traits with only one measurement, only cytotype was included as a factor in the analysis. Triploid plants differed from diploids and/or tetraploids for almost all traits.

Size Neither root: shoot mass ratio, or root mass showed a significant difference among the three cytotypes (Table 1). Similarly there was no significant difference for total stem length or stem number. However, diploids were significantly different than triploids and tetraploids with respect to shoot mass. In contrast, tetraploids were significantly different than triploids and diploids in terms of internodal number and distance. Triploids have the largest average shoot weight, but the smallest average shoot length, and an intermediate, though more similar to the larger tetraploid, stem girth. Overall, triploids are more similar to diploids, but only by a margin of one trait (Table 1).

Table 1. *Fitness*

Trait	**2x** (cm)	**3x** (cm)	**4x** (cm)	**Significance** (*P*-value)
Total stem length	51.500 A	39.333 B	42.559 AB	NS (0.0606)
Root: Shoot	0.212 A	0.144 A	0.129 A	NS (0.3504)
Shoot mass	1.405 A	2.201 B	2.053 B	<0.0001
Root mass	0.222 A	0.325 B	0.259 AB	NS (0.0879)
Internodal number	12.688 A	11.400 A	6.733 B	<0.0001
Internodal distance	4.001 A	3.467 A	5.132 B	<0.0001
Stem girth	1.922 A	2.607 B	10923 B	0.0020
Stem number	1.250 A	1.600 A	1.308 A	NS (0.4826)

Morphology No significant difference was evident for leaf color or the measurement from the base to the tip of a leaf (Table 2). Diploids were significantly different from triploids and tetraploids for traits such as measurements of base to sinus, lobedness, and petiole length and stem girth. Tetraploids differed significantly from triploids and diploids for the following traits: maximum leaf length and width, and leaf number. For the base to lobe measurement, diploids differed from tetraploids, but triploids did not differ significantly from either. Ultimately, triploids were more similar to diploids, but once again only by a difference of one trait (Table 2).

Flow Cytometry All but one purported triploid individual, I 103-G-6, were confirmed as triploids by flow cytometry (Table 3).

Table 2. *Morphology*

Trait	**2x** (cm)	**3x** (cm)	**4x** (cm)	**Significance** (P-value)
Base to tip	3.467 A	3.493 A	3.387 A	NS (0.5761)
Maximum leaf length	4.406 A	4.515 A	4.859 B	0.0152
Maximum leaf width	4.795 A	4.986 A	5.390 B	0.0050
Base to sinus	1.267 A	2.097 B	2.249 B	<0.0001
Base to lob	2.568 A	2.276 AB	2.904 B	0.0022
Sinus: Lobe (lobedness)	0.498 A	0.762 B	0.778 B	<0.0001
Leaf number	12.958 A	10.733 A	7.462 B	0.0040
Leaf color	5.420 A	50327 A	5.490 A	NS (0.1022)
Petiole length	3.748 A	5.206 B	5.724 B	<0.0001

Table 3. 2C DNA content for diploid, triploid and tetraploid ivies

Origin	Genotype ID	2C DNA content avg. (range)	Ploidy
St. Edwards State Park	2x St. Edwards	3.02 (2.86, 3.12)	2x
St. Edwards State Park	3x St. Edwards	4.73 (4.59, 4.99)	3x
St. Edwards State Park	4x St. Edwards	6.75 (5.86, 7.22)	4x
St. Edwards State Park	Lake 104 2 of 5	5.04	3x
St. Edwards State Park	I 103-G-6	6.46	4x
St. Edwards State Park	I 103-G-5	4.72	3x
St. Edwards State Park	I 103-G-2	4.80	3x
St. Edwards State Park	I 103-G-1	4.71	3x
St. Edwards State Park	Lake 102	4.90	3x

Discussion

Triploid ivy from St. Edwards does not consistently deviate, in morphology or fitness, from sympatric diploid and tetraploid individuals. In the majority of traits analyzed, triploids were found to be more similar to diploids, but this does not mean that triploids would necessarily be substituted successfully into the commercial market. Traits must be examined individually for specific advantages or disadvantages. Additionally, the data indicate that diploids and tetraploids are significantly different in almost all traits measured. This would suggest that there is significant morphological and size variation in the horticultural stock, and thus may facilitate the addition of a new morphology.

Size Triploids and diploids have more leaves than tetraploids but also smaller leaves in terms of width and maximum length. Tetraploids are often used in landscaping because their broad leaves form a denser ground cover and can provide better erosion control. They are typically planted by highway maintenance departments, especially in the Northwest. While triploids are more similar to diploids in terms of length and width, triploids are more congruous with tetraploids for lobedness, which would suggest a larger surface area. This along with more leaves on average, may mean that triploids are at least comparable to tetraploids for certain applications. Triploids also share similar values for shoot mass (3x>4x) and girth with tetraploids. Triploids have the largest average shoot mass, but the smallest average shoot length, and an intermediate stem girth. It may be possible to explain this apparent contradiction by the fact that they do have longer petioles and more leaves, suggesting a higher leaf and/or petiole: stem ratio, which could be desirable especially in landscaping situations where leaf cover is important. It is important to consider water requirements with the increased leaf mass, as this might be a disadvantage in water stressed regions. On the East Coast, *H. helix* (2x) is commonly used for groundcover because it is hardy to the cold. If triploids were similarly hardy they might be a more desirable option in the region because they would combine advantageous traits of tetraploids (e.g. larger leaf cover) with those of diploids (e.g. increased cold tolerance).

The lack of significance for the root : shoot ratio with respect to ploidy would suggest that shoot mass is correlated with root mass, providing a useful above ground indicator for root mass for all cytotypes. This correlation does not hold true for shoot length or girth however, which would be more convenient indicators.

Morphology Triploids differ equally from diploids as tetraploids for most morphological traits. No difference, however, was evident in leaf color or base to tip measurements between the three cytotypes. Triploids are not as lobed as diploids, but share many other traits with diploids. In contrast, triploids are only similar to tetraploids for the base to sinus distance and petiole length. Triploids appear to be true morphological hybrids. It is unknown whether horticultural consumers prefer diploids or tetraploids based purely on morphological differences. However, the fact that two distinct morphologies have a significant horticultural presence would suggest that customers would be open to a new morphological variant.

Flow Cytometry The unexpected result for 1103-G-6 requires further testing to determine if the value for 2C DNA content is correct, and is in fact tetraploid. The quality of the control DNA used in this run was questionable, so the inconsistency in data between the expected and observed ploidy level could be spurious. This individual, 1103-G-6 was considered triploid for the purposes of data analysis.

It is important to mention that the data and conclusions made from this study are specific to the *Hedera* populations in St. Edwards State Park. These results should serve to inform and direct further studies comparing the traits of *Hedera* species and cytotypes.

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