A Stanford-Brown iGEM 2017 Human Practices Report: Contrasting Terrestrial and Extraterrestrial Rubber Pipelines, and a Synthetic Biological Solution

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Abstract

Shoe soles, refrigerator gaskets, car tires, vibration insulation on space shuttles - all of these technologies, though wildly different in their applications, are interconnected through the use of rubber. Due to the its numerous applications and unique properties, rubber has become a material which is both a staple of a variety of industries, and is inextricably tied to our daily lives. Despite numerous applications, even the most useful of materials faces limitations. While seeking to utilize rubber to its maximum material potential on earth and avoiding environmental concerns in doing so biotechnology companies, tire manufacturers, and iGEM teams alike have all sought to address production concerns facing the rubber market. As the location where production is staged changes from terrestrial to extraterrestrial, however, so do the concerns facing the industry. Synthetic biology is a creative solution, and perhaps the best for a low mass, high efficiency alternative for both conventional and projected rubber applications in space.

What does the rubber market look like on Earth?

Most of the rubber used in industry today starts its life cycle in the lactifier cells of the *Hevea brasiliensis* rubber tree, beginning as an emulsion of milky latex sap. Biologically, latexes have a function in herbivore defense, while laticifers are a dumping ground for metabolic by-products or reservoirs of biosynthetic materials. All latexes are emulsions, aqueous suspensions of insoluble materials which can include alkaloids, terpenes, resins, phenolics, proteins, sugars, and long-chain hydrocarbons.[1] Though *Hevea brasiliensis* originated in Brazil, currently, “Asia accounts for 97 percent of the world’s natural rubber supply, with the vast majority coming from Thailand (31 percent), Indonesia (30 percent), and Malaysia (9 percent).”[2]

To harvest raw rubber latex, tappers collect this emulsion through cutting thin, diagonal channels in the trunk of the rubber tree. With a small tap secured in place, the liquid latex slowly spills downward into a pail for collection. Following this step, the liquid latex will be coagulated in a pan, the water rolled out, and the rubber put through a set of rollers. Prior to transportation, the tappers may also smoke the sheet of rubber over a fire. In some cases, depending on the buyer, the latex emulsion might be transported in a liquid state instead of this dried form. In total, “annual yields under tapping for 2-5 days a week on 1-2 half spiral cuts are 500-600 kg per hectare.”[3]

Although individual rubber tappers are the direct mechanism of rubber latex collection, the industry’s organization structure is complex. The rubber industry has several production tiers, ranging from the predominant, independent smallholdings to vast estates. As C. Barlow of Australian National University explains,

> “Estates are defined as units of 25 or more planted hectares under one management. The bigger estates in particular are operated in a well-defined hierarchical organization of managers, supervisors, and paid workers, which is characteristic of plantation enterprises throughout the world. Smallholdings are defined as units less than 25 hectares, and are family farms whose output may be supplemented by areas of other crops, especially padi. They typically comprise 2-3 hectares of planted rubber. which is often divided into several parcels. They are generally worked by family members, although larger holdings also employ labourers.”[3]

Smallholdings generally receive smaller payment per kilogram of rubber produced, due to transportation and other production constraints limiting their rubber quality. These individuals are also affected more severely when the rubber market fluctuates, due to their increased sensitivity to the market pricing and currency appreciation.

Whether sourced at an estate or in an independent smallholding, the raw rubber eventually reaches manufacturers. The chief manufacturing consumption of natural rubber is in Europe, North America, Japan, and China.[3] China is the highest consumer, with tires being a prevalent and pervasive example natural rubber being used in industry. As displayed in Figure 1, natural rubber typically accounts for "around 28% of a conventional automotive tyre compound by weight, with the remainder comprised of synthetic rubber (28%), carbon black (28%) and various functional agents (16%)."[4] A question might arise as to how an emulsion of latex sap transforms into a substance durable enough to support a vehicle. This happens in the manufacturing process when the natural rubber undergoes a process called vulcanization, by which chemical impurities (traditionally sulfur, though many others are considered trade formulations) are added, and the material is heated. Cross-linking occurs at the molecular level by this process, which is what ultimately lends rubber the favorable material properties of elasticity and compressibility for which is so often used. Natural rubber, however, is not the only type of rubber used in manufacturing, as is illustrated with the diagram below.

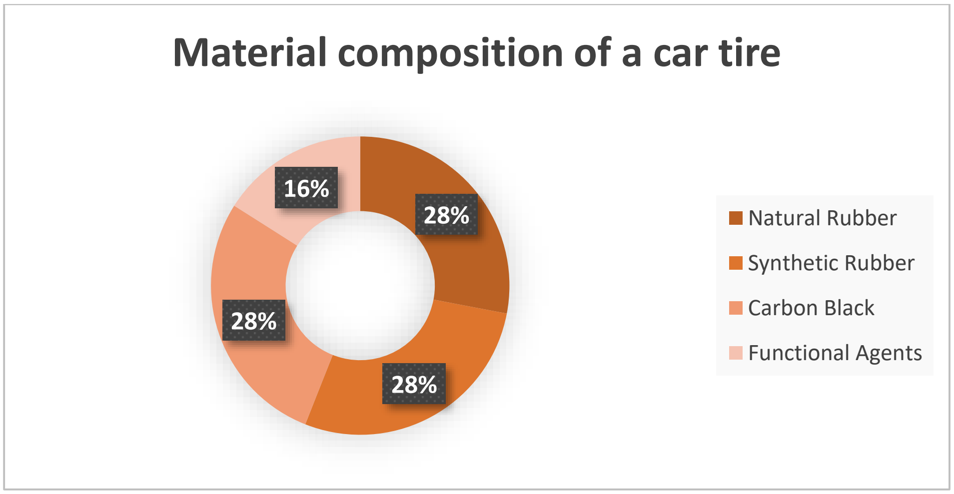

Figure 1: Material composition of a car tire.[4]

The second type of rubber used in manufacturing is referred to as "synthetic." Rather than *Hevea* latex, manufacturing synthetic rubber utilizes petrochemical products from refining petroleum to create synthetic polymers. Synthetic rubber is appealing due to its superior weathering capabilities and the ability to alter or customize properties through chemical means. Generally, however, manufacturers do not favor one rubber type exclusively over the other. While today it is possible to "produce synthetic polyisoprene that has physical properties similar to those of natural rubber with a purity of 98 to 99%, ... the stress stability, processability, and other parameters of synthetic polyisoprene are still less satisfying than those of natural rubber."[5] While industry figures vary regarding the exact proportions of synthetic and natural rubbers that are utilized today - some accounts quote a 60% synthetic foothold and others as high as 70% - it is because of these variances that natural rubber market still maintains a strong market share. In fact, based on a January 2017 report by the Rubber Economist, "consumption is estimated to have increased for NR (natural rubber) and decreased for SR (synthetic rubber); sharper growth of NR over SR is expected to remain. SR share is estimated to have fallen sharply this year, and the trend may continue into 2017 and 2018. Slow growth in world NR output is estimated to have continued from last year into this year, but a more rapid growth is expected."[6]

With projected growth anticipated in the natural rubber market, it should be noted that *Hevea brasiliensis*, while dominant in quantity of production, is not the only source of natural rubber latex. For example, *Taraxacum kok-saghyz*, the Russian dandelion, demonstrated marked success when it was used by the Soviet Union as a domestic source of rubber during the second World War.[7] In the contemporary era, *T. kok-saghyz* is again being evaluated by multiple sources, this time alongside *Parthenium argentatum* Gray (guayule) as another potential alternative.

Which "isoprene" reigns supreme?

With two types of production vying for shifting industry shares, one might question whether or not a champion is to emerge in the foreseeable future. Colleen McMahan of the USDA, and a researcher in the synthesis of rubber, had one notable comment about this conflict during a personal interview with Stanford-Brown 2017. "If we were able to replace it, it would've been done already."[8]

Were the properties of natural rubber able to be replicated perfectly, synthetic rubber would have already dominated the industry. Instead, either substance is used where it is better suited, or blends of both natural and synthetic rubber are balanced to yield products with useful, adapted properties. Utility is determined through application, and both types of isoprene have applications relevant to their unique properties, as is shown in table 1. Neither type stands to be replaced exclusively by the other given their unique distinctions in terms of production source, location, monomer type, and material properties.

Table 1: Differences between natural and synthetic rubber.[19]

Differences between natural and synthetic rubber		
Factors Assessed	**Natural Rubber**	**Synthetic Rubber**
Production Source	Biosynthesis in *Hevea* or other plants	Crude oil by-products or emulsion polymerization
Production Location	Geographically limited based on production species	Not limited geographically
Monomer type	cis-1,4-isoprene	Varies with type
Properties	Difficult to alter	Chemically adjustable

What problems face the rubber industry?

The natural rubber industry is impacted drastically by conditions of currency appreciation, and changing environmental conditions. Of these concerns, the latter has the potential to have a drastic impact on both the natural and synthetic rubber markets. Disease, lack of biodiversity, and resource costs threaten the natural rubber market, whether on a smallholding or an estate scale. With the synthetic market, there is growing concern about the long-term scarcity of petroleum-based resources.

South American Leaf Blight, though primarily a threat in the native Amazon of the *Hevea* tree, has potential for grave consequence. Caused by the ascomycete *Microcyclus ulei*, this disease is charged with causing poor latex yield and the untimely failure of plantations across South America. *M. ulei* strikes many trees before they reach physiological maturity. The disease is still restricted to its continent of origin, but its potential to be distributed around the world rises with every transcontinental airline connection that directly links tropical regions. “Even the use of modern systemic fungicides and use of greatly improved application techniques have failed to prevent large losses and dieback of trees.”[9] It would take but one mishap for a transmission event, perhaps from an uncleaned cargo ship, to inadvertently transmit to another location, where a virgin population of *Hevea* awaits, ready to cripple an industry as consequence of a new infection.

Clearing massive swaths of land in favor of planting rubber trees concentrates such a virgin population into a geographically confined space, which has little diversity to combat such an infection. This is another major concern. Striking a balance of genetic resistance and adequate production is difficult when it may take decades for a tree to reach maturity. In the broader scope, this is a general agricultural issue, also affecting genetically modified crops which would also be at risk of extinction due to one wayward pest or infection.

No production scale venture comes without resource cost, and in the case of the natural rubber industry, the costs of water and raw energy are significant. Watershed function can be impeded when land is uprooted in preparation for an anticipated *Hevea* plantation. “With respect to hydrology, rubber is blamed to be the cause of the dramatic downward trend in fog frequency between the mid-1950s and the mid-1980s. Research has also shown that surface runoff increased by a factor of three, and soil erosion increases by a factor of 45 as a result of conversion from tropical forest to monoculture rubber in Xishuangbanna.”[10] Additionally, when considering financial expenditures, 75% of the cost associated with rubber collection is attributed to energy. 10% to capital cost, with another 15% attributed to maintenance.[11] Consider the aforementioned processes involved post-tapping, notably the extrusion of the rubber on rollers and the vulcanization process required to lend rubber the desired properties. Among the rubber product manufacturing processes, “the milling process, extrusion process and rolling process have a relatively higher electric power consumption which is more than 50% of the total consumption, while the vulcanizing process uses up 80% more or less of the total consumption.”[11] Until another processing step can be substituted in place of vulcanization, that cost is unavoidable. Lest numerous smallholders world-wide lose their livelihoods, the other energetic costs can only seek to be improved upon, as well, rather than eliminated altogether.

Considering synthetic rubber, and the requirement of petroproducts as requisites to synthesis, an entirely different concern emerges. Petroleum is regarded as a dirty, inefficient fuel. In the collection of oil, environmentally concerning processes such as fracking are often required. “There is still a lack of information on exposure to natural and added chemicals, and their fate and ecotoxicity in both the discharged produced and flow-back waters. [For example,] geogenic contaminants mobilized from the coal seams during fracking may add to the mixture of chemicals with the potential to affect both ground and surface water quality."[18] Many figures are already assessing the long-term viability of such technologies, notwithstanding the fact that on Earth, we are already suffering climatic effects from the releases of greenhouse gases, petro-products, into the atmosphere.

Where does Synthetic Biology fit in?

Both industry leaders and iGEM teams alike have tackled rubber production's associated issues. Though primary industry approaches centered around the production of isoprene through synthetic biology, iGEM teams have taken a more varied approach, tackling issues ranging from production to degradation of rubber.

Industry

The two biggest industry investments in the production of rubber through synthetic biology can be observed in the partnerships between Genecor and Goodyear, and Amyris and Michelin. In the first partnership, Genencor, a Danisco Division, produced isoprene monomers (the most basic units of rubber latex) through microbial fermentation via a collaboration with The Goodyear Tire Company. The success of this venture lead to patents being filed in 2010 (publication numbers US 8546506 B2, EP 2513021 A2, EP 2582649 A1, and US 8507235 B2 respectively), for the now trademarked BioIsoprene™. Amyris and Michelin also partnered in a similar venture, as was announced by Chemical and Engineering News in October of 2011.[12] Notably, patent US 20130030227 A1 was documented as a result of this partnership.

Timing was critical for both of these partnerships, as was confirmed through speaking to Kevin George, a senior research scientist at Amyris. When questioned by the Stanford-Brown iGEM team about the influence of petroleum price fluxes and the 2008 recession influencing research into synthesis of isoprene, George stated that "the first wave of hype for synthetic biology was regarding replacement for high volume chemicals (gas, diesel, jet fuel) at commodity scale. Interest waned as oil prices went down. It is challenging to scale biology cheaply."[13]

Caroline Peres, a Principal Scientist of Microbial Physiology and Fermentation at DuPont, echoed this sentiment during a personal interview, also clarifying several points about the Genecore/Goodyear collaboration in the production of isoprene. When questioned regarding reasons to scale synthetic production of isoprene rather than pursuing traditional methods, she spoke to the fact that "we want to have another source, not to replace the market, as a goal. There's enough space on the market for more than one player."[14] She also went on to bring up the critical environmental concerns when *Hevea* is relied on exclusively: climate change, pests, and global climate change. Even with the nature of rubber contrasting the traditional high-cost, low-quantity cosmetic additives such as squalene which Amyris produces, production concerns do still require redress. This becomes all the more critical were someone interested in scaling synthetic biology for space applications.

Even traditional production in space is not a technology of the distant future, to that point. Made in Space, a company known for their 3D printers designed for use in space and other extreme environments, took a significant leap into the realm of manufacturing extra-terrestrially. Though their manufacturing processes aren't focused on synthetic biology, harnessing the effects of microgravity can prove highly beneficial in manufacturing processes. On Earth, optical fiber suffers signal loss due to the presence of small crystalline flaws from the manufacturing process. However, in space, "ZBLAN optical fiber can be produced without these crystals, providing superior data transmission capabilities compared to both Earth-produced ZBLAN and traditional silica fiber optic lines. … This microgravity-produced fiber has numerous applications, including transatlantic telecommunications, high-speed internet, lasers, as well as enhancing technologies in space.”[15] With traditional manufacturing already demonstrating that microgravity can lend significant benefits to materials produced in space, one can begin envisioning how biologically engineered machines might fare and even be enhanced through a similar production scheme.

iGEM Teams

iGEM teams have tackled the problems of rubber production from multiple angles, each crafting a unique solution for their respective focuses (fig. 2). SDU-Demark 2013’s Bacteriorganic Rubber project (http://2013.igem.org/Team:SDU-Denmark) focused on the generation of rubber in bacteria, taking advantage of the *Hevea* HRT2 gene to allow for polymerization of IPP and successive DMAPP molecules (both of which are basic isoprene units) to form rubber latex. This team also created constructs to increase DMAPP and IPP production, which would otherwise limit the rate of rubber production. In 2015, Brasil USP (http://2015.igem.org/Team:Brasil-USP) tackled the issue of rubber degradation. The team focused on the use of a pretreatment with *Acidithiobacillus ferroxidans* to devulcanize rubber, followed by an enzyme treatment with rubber oxygenase A (RoxA) and Latex clearing protein (LCP) to degrade the devulcanized rubber. Stanford-Brown 2016 (http://2016.igem.org/Team:Stanford-Brown/SB16_BioMembrane_Latex) then further expanded and refined the work of Denmark 2013 with their addition of a second *Hevea* gene in a construct and a secondary construct to increase the quantity of rubber produced.

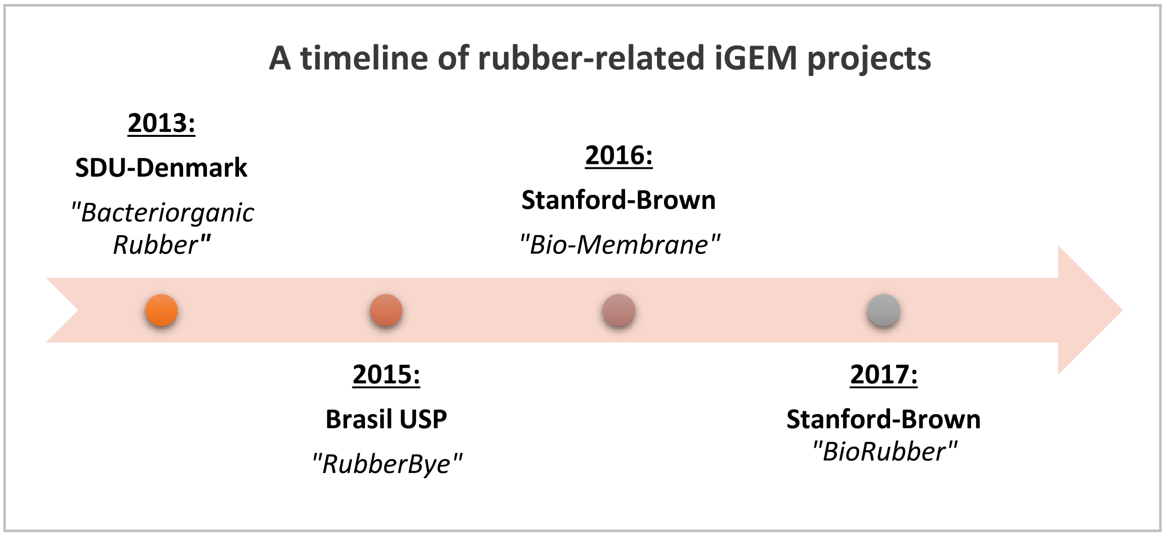

Figure 2: A timeline of rubber-related iGEM projects. Image by the author.

With a new cycle of iGEM competition, Stanford-Brown 2017 sought to tackle the issues of generation and degradation in a novel and more efficient way. While Stanford-Brown 2016 did successfully synthesize and extract latex, their methods had two points to be improved upon.

Foremost, latex extraction required lysis of the genetically modified *E. coli.* While bacteria are easily self-replicating, lysis is unfavorable in the conditions of space where resources to re-engineer the *E. coli* are nil in the event of any problem. Lysis also requires additional reagents contributing to higher up mass, and consequently, higher mission cost. Also, a product of intracellular synthesis, the quality of latex produced was suboptimal. Though the extracted product did demonstrate both the scent when burned and some material properties latex, the texture was grainy and seemed limited in its manufacturing potential. In seeking to address this issue, the BioRubber team of Stanford-Brown 2017 sought to create a fusion protein with the cellular machinery of latex polymerization (*Hevea's* cis-prenyltransferase enzyme, or cis-PT) and the well-characterized Outer Membrane Protein A (ompA), as is modelled in figure 3. Though this project was ultimately stalled at the end of summer due to time restraint, it is still certainly a promising solution in effort to alleviate waste in bacterial latex production.

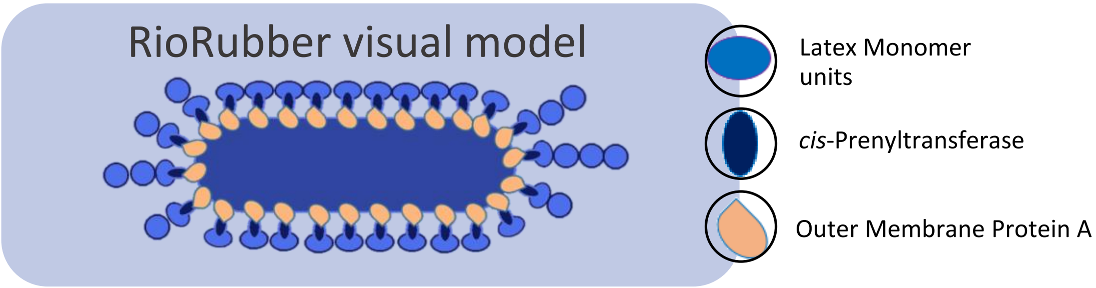

Figure 3: BioRubber visual model. Image by the author.

Following the thoughts of 2015 Brasil USP, Stanford-Brown 2017 knew we wanted to thoroughly treat the latex project. Oliver Morton, a science writer known for his work in *Discover, National Geographic, and Nature*, brought to our attention at the beginning of the summer the fact that, "No pathway is good without a reverse."[16] Cyclical is better. With that in mind, we also considered ways to cross-link produced liquid latex, and methods to degrade a product. What we envisioned, merging this half of the project with our synthesis component, was an ecosystem. Aboard the ISS, in a distant habitation module on Mars, or even in a lab on Earth, one might discard a latex glove into a culture, which could then degrade the latex into a material then useable in another culture. Once the secondary culture was given its feed of recycled latex, a secondary process could be executed to cross-link the liquid latex into a useable, solid form. Broken latex components could also be recycled, providing ample fuel to create new components as needed.

Design considerations for both halves of our BioRubber project were heavily influenced by the experts we spoke to in industry and academia, and our research of the present rubber market. Industry scientists quickly spoke to the explosive hazard associated with managing isoprene, which prompted careful consideration to how we might avoid the gaseous material. Experts including Jim Head and our primary advisor Lynn Rothschild also prompted us to consider the critical needs for safety, efficiency, and versatility for anything to be used in space. With evaluation of the rubber market, we began questioning exactly what would happen if the market were translocated to a new location, with radically different resources. In considering this, we realized that synthetic biology had a niche which had immense potential and great need beyond the confines of our planet.

Space Applications – An Unmet Need

With the work of terrestrial industry and iGEM teams seen as a proof of production capabilities, and Made in Space demonstrating extraterrestrial manufacturing outside of a critical need to do so, a possibility of new potential emerges. Any time a mission is sent into space, astronauts are knowingly separating themselves from an environment of ample resources. On earth, if something is broken, it is of little inconvenience to drive to a hardware store to retrieve a replacement. Services such as Amazon Prime can bring even the most diffuse of items to one's doorstep in a matter of days. In the isolation of space, however, the story is much different. Preparations are critical and back-ups essential, lest an entire mission be compromised with the failure of one part. Without a replacement, or having means to generate one, astronauts are at the mercy of the vacuum. This need is further emphasized with the potential for colonization missions being launched in the foreseeable future. In the context of both short and long-term missions, the need for high value-to-mass materials cannot be understated.

In contrast to the restrictions imposed in space, the lack thereof should also be acknowledged. Without established economy in space, there is virtually no competition - nor limit - to the potential for synthetic biology in space applications. Much in the way of conventional industry, such technologies would require refining and significant testing to be streamlined. However, it should be acknowledged that in terms of low-mass, low-cost, self-replicating alternatives to conventional materials, those generated from genetically engineered machines hold innumerable potential in their diverse array of applications, and there is ample time to develop solutions.

In making such a proposition, it should be known that despite a limitless frontier, there are two primary restrictions: resource requirements, and planetary protections. In terms of resource requirements, streamlining microorganisms for material production will take time and funding. Microorganisms, like any *Hevea* tree on Earth, require sustenance to produce their respective materials. Even the most ambitious aquaponic-inspired system of bacterial cultures feeding other cultures may well have its limit in solving this issue. Though this is not the problem Stanford-Brown iGEM set out to solve this year, it is a concern that is entirely legitimate and would require forethought and a creative solution.

Another matter entirely, planetary protection is also of utmost concern. It is true that sending cultures with the express purpose of producing or degrading materials for interplanetary immigrants will likely surpass the 300,000-spore limit imposed by Article IX of the Outer Space Treaty of 1967. Mars Exploration Rovers 1 and 2 were held strictly to this requirement, with the "aim to protect other solar system bodies from Earth life and ... Earth from extraterrestrial life that may be brought back by returning space missions."[17] Were cultured materials meant to be kept exclusively in human-occupied structures, contingency plans still require keen forethought and delicacy as to the containment and potential ramifications should any organisms be released.

Conclusion

No industry is without challenges. The rubber industry, both in terms of resources and environmental effects, is no exception. With ample attention being given to the development of synthetic biological tools on earth, particularly in the content of the iGEM competition, however, the argument can be made that the groundwork for yet-unseen technological applications is being established. The sky is not the limit when such technologies are considered as tools for space exploration. Escaping conventional industry limitations in favor of an industry yet established holds immense potential for synthetic biology.

Furthermore, while a low-cost, competitive market and challenges of scale pose an intimidating challenge, technologies such as BioRubber could both fulfill an unmet need in the future of space exploration, and could prove useful as an alternative material when the Earth's own natural resources are pushed to their limits.

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Concluding Words from the Author

Despite my primary interest in biology at Brown University, I also have an interest in political science. Specifically, I am interested in how science policy is influenced when individuals convey complex messages in terms that even a lay-person can understand. When designing this report, I wanted to put forward something that fleshed out a story interweaving the rubber market, project applications from past iGEM teams, and the significance of synthetic biology for space exploration in a cohesive way. I wanted this story to be clear and educational, albeit also accounting for technical details, allowing even a non-science reader the chance to glimpse the story Natalie and I did while conducting our human practices research for the team this year. Thank you for reading.

\- Sierra Harken