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Article

A bibliometric analysis of the use of biomimetic silk conduits for treating peripheral nerve injuries

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SUMMARY

Peripheral nerves are critical because they function as a relay between the brain, spinal cord, and the rest of the body. However, because the peripheral nervous system is not protected by bones, it is vulnerable to injuries. Severe peripheral nerve injuries (PNIs) are categorized by the presence of nerve gaps – spaces between two ends of a transected nerve. Common treatments for such injuries are nerve grafts or synthetic conduits, but these treatments have various limitations which have led to research into the development of silk conduits for PNIs. In order to examine this novel research field, we employed a bibliometric analysis, a form of analysis where statistical methods are used to interpret previously collected data. We created and applied a 3-analysis method that provided both quantitative and qualitative information. The methodology was developed to answer our three research questions: 1) How has the field of peripheral nerve regeneration conduit research, and its subfields, grown in the past 20 years? 2) What are previous successful and unsuccessful approaches? 3) What are possible areas for future studies? The growth analysis we conducted showed a clear increase in total number of papers published about conduits per year, especially for silk conduits. Our analysis also revealed that silk conduits performed almost as well as nerve grafts and identified some promising properties for further *in vivo* testing, including biocompatibility, biodegradability and the ability to bridge any length of gap.

INTRODUCTION

The peripheral nervous system (PNS) consists of the nerves outside of the central nervous system (the brain and spinal cord). The main function of the PNS is to serve as a relay between the rest of the body and the brain and spinal cord (1). There are three types of peripheral nerves: sensory, autonomic, and motor (2). These nerves allow us to feel pain and other sensations, control critical involuntary functions such as heart rate, and stimulate movement (2). The PNS, unlike the central nervous system, is not protected by bones (vertebrae and skull), thus making the PNS more susceptible to injury (1). Damage to a peripheral nerve is known as peripheral nerve injury (PNI) and is categorized in a 5-degree classification system (3). Fourth- and fifth- degree injuries always require surgical intervention, either through grafting or implantation of conduits (3). In these injuries, there is severe damage to the axons and surrounding tissues that prevents natural nerve regeneration, and specifically in fifth-degree injuries, the nerve is completely transected, creating a nerve gap (3).

The body has natural neurochemistry mechanisms to maximize nerve regeneration, but these processes do not always lead to full regeneration before nerve death or full functional recovery of the nerve (4). The largest obstacle is gap length. Gap length is the measurement of how many millimeters of the nerve has been damaged and needs to heal (5). Longer gap lengths are detrimental to effective nerve regeneration, leading to a lower rate of successful regeneration (5). With the average regeneration rate, nerve injuries as short as 10 cm can take upwards of 100 days to heal, and further, some proximal nerve injuries can involve up to a meter of damaged nerve, which would take anywhere from 2-3 years to heal to only partial functional recovery (6). Additionally, some peripheral nerves, such as motor nerves, have a short time limit in which they must heal before they die (7).

The need for nerve regeneration optimization has led to the development of treatments for PNI, of which the two most common are nerve grafts and nerve conduits. A nerve graft involves taking a nerve from a different part of the body or a donor (human or other), where the nerve does not play a critical role, and use it to bridge the gap in the damaged nerve (8). Autografts and allografts are the two forms of grafts used. Autografts are nerve grafts from another part of the same individual's body, but this method is problematic if the nerve gap is too large (9). Allografts come from a non-human donor, but the concern of biocompatibility is paramount, and this type of graft may result in more damage than healing if the graft is rejected (9).

Nerve conduits are used as alternatives to nerve grafting (10). They are inserted between the proximal and distal stumps of the damaged nerve and act as a guiding channel for regrowth (10). In addition to providing support for axon regrowth, they protect the healing nerve from surrounding scar tissue and inflammation (10). There are two main categories of conduits: biological and synthetic conduits. Biological conduits are made from materials such as arteries, veins, muscle, and even umbilical cord vessels (11). Synthetic conduits include both nondegradable and degradable conduits. Nondegradable conduits are commonly made from silicon, while degradable conduit materials include collagen, chitin, various forms of acids, and hydrogel (11). Despite the prevalence of these treatments, there are complications that arise. With nondegradable conduits, there is the requirement for a second surgery for removal, which causes additional pain and adds risk (11). With biological and degradable conduits, the main concerns are biocompatibility and the rate of degradation of the material. The body should not produce an immune response to the material of the conduit, and if the degradation rate is too fast, it may lead to swelling and inflammation; however, if degradation is too slow, it may lead to nerve compression (11). Lastly, there is an upper limit of a three-centimeter nerve conduit length, so PNIs longer than that length have completely insufficient treatment options (12). In an attempt to create the ideal nerve conduit that can address these issues, research has led to the biomimetic use of silk for nerve conduits.

Biomimetics is the development of synthetic systems or products that mimic biological structures, processes, or properties of biologically produced substances (13). Scientists in this field study topics ranging from bacteria to derive inspiration for biological motors, to birds to improve aerodynamic lift, and even biological systems to develop better mechanisms for self-healing. (13). Inspiration for biomimetic concepts and technologies can be found from any biological structure or mechanism in nature, such as silk (14, 15). Silk, defined as a "protein-based fiber-forming material spun by living organisms" was first used by the Chinese in 4000 BC and has since been coveted as a luxurious material in fabrics (16). Recently, silk has been subject to increased interest in the field of biomedical applications due to its incredible potential as a biomimetic material. The most prevalent example of silk's potential being harnessed would be the silk suture, which has been used in the biomedical industry for more than 100 years (17). However, the interest in silk and its applications has spread from purely biomedical, to areas such as bioengineering for tissue regeneration, highlighting the versatility of this material (18).

The motivation for this interest in silk is founded on three main properties: mechanical characteristics (strength and elasticity), biodegradability, and biocompatibility (19, 20). Biodegradability and biocompatibility are essential aspects to consider for biomedical materials. A biocompatible material is one that when inserted into a host, does not result in a sustained inflammatory or toxic response that would be harmful to the host. Silk has been proven to be biocompatible when studied both *in vivo*, in different organisms including rats and dogs, and *in vitro* (20). Additionally, a biomedical material should be biodegradable, meaning that it should be able to last in the host's body for a sufficient period of time then be able to break down naturally in the body, without the need of surgical intervention and removal. Most importantly, when such a material begins to degrade, the smaller compounds into which it is broken should be non-toxic, meaning that they do not induce an immune response, and be easily metabolized and cleared from the body (19). Silk biomaterials are biodegradable both *in vivo* and *in vitro*. Silk is digested by various enzymes in the body, and it is broken down into amino acids that are absorbed safely and easily by the body (19).

However, properties of silk can vary depending on whether the silk is from spiders or the moth *Bombyx mori*, also known as the silkworm. Both types of silk share many important features, such as molecular structures, but there are also differences between them that may have implications for various products (18). Moth silks all have the same fundamental design, but because each individual spider is able to produce numerous types of silks, spider silk covers a large range of design types (18). Perhaps the biggest differences between the two types of silks are the strength and flexibility of the fibers. Silkworm silk is very flexible, but weak compared to spider silk, which requires extremely high strain to break, but is less flexible (18). The usage of silk in the development of nerve conduits and the facilitation of nerve regeneration is a critical area of research for two main reasons. Firstly, spider silk specifically has been shown to have properties that enhance Schwann cell migration and axonal regeneration, thus speeding up the nerve healing process (21). Secondly, silk is a biocompatible and biodegradable material, two criteria that should be met for the ideal conduit. These combinations of features mean that silk has incredible potential to revolutionize how nerve injuries are treated and how long it takes to regenerate nerves, increasing the likelihood of proper function returning to damaged nerves (22, 23).

The goal of this paper is to use statistical analysis to provide a comprehensive overview of the conduit field and to answer the following research questions: 1) How has the field of peripheral nerve regeneration conduit research, and its subfields, grown in the past 20 years? 2) What are previous successful and unsuccessful approaches? 3) What are possible areas for future studies? We hypothesized that the field of peripheral nerve regeneration conduit research has grown steadily over the past 20 years, and specifically, that the use of silk-based nerve conduits has seen immense growth because the silk-based conduits show significantly more success than traditional approaches. Overall, we found that there was a statistically significant growth in the publication of silk conduit focused papers, but more research and development is needed to realize their potential to perform better than current treatments.

RESULTS

Growth of the Field (Analysis 1)

Analysis 1 was designed and executed with the first research question in mind. The methodology for Analysis 1 implemented a control search phrase ("peripheral nerve" repair conduit), along with various treatment words (vein, artery, hydrogel, polymer, ligament, collagen, silk, "spider silk," and "silkworm silk"), that were typed into Google Scholar. We repeated this for each year starting in 2000 for each treatment, including just the control phrase. Our output measure was the number of total papers published that year (listed at the top of the page).

We found a clear increase in total numbers of papers published about conduits per year, but also a specific growth in the number published about silk conduits (**Figure 1A**). Silk was the only treatment that showed an exponential growth curve, as compared to the other treatments which either seem to be stagnant (artery, ligament, and vein) or demonstrate linear growth (hydrogel, polymer, and collagen) (**Figure 1B**). Silk saw a proportional growth of close to 20% over the 20 years, the highest out of any of the other treatment groups (**Figure 1B**). We also found that both spider and silkworm silk, though seeing various fluctuations in terms of proportion to total silk papers published, have seen significant increase in proportion over the past 20 years (**Figure 1C**). In 2000, the proportion for both silkworm and spider silk was close to 0, but by 2020 the proportion was above 0.08 for both (**Figure 1C**). Our analysis revealed that spider silk initially had the fastest growth, but by 2005, silkworm silk had also started to gain interest. By 2020, neither spider nor silkworm silk seemed to have an increased preference in the literature (**Figure 1C**). Overall, the results

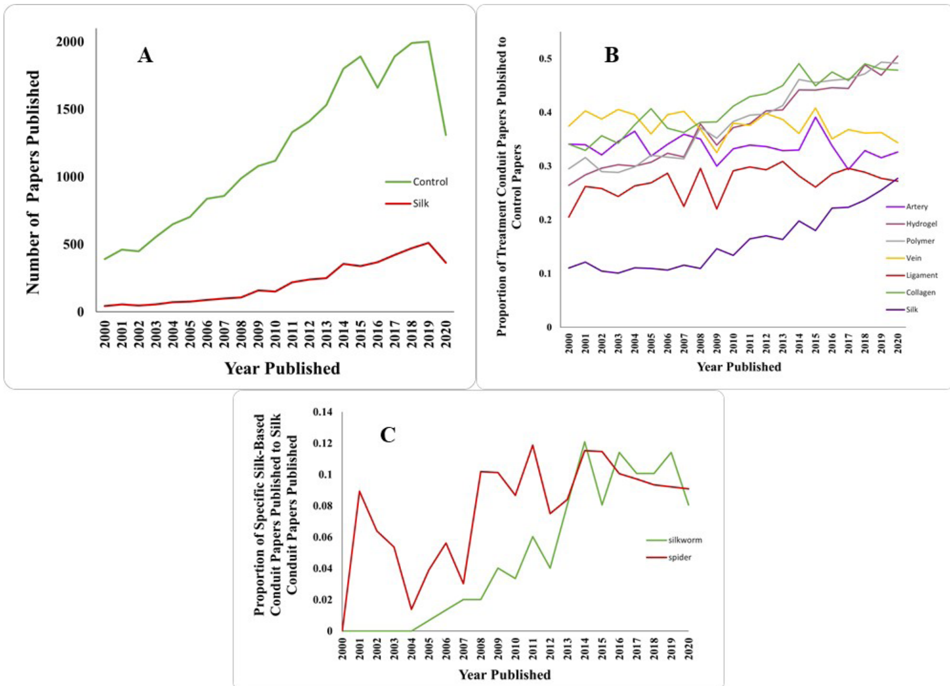

Figure 1: Growth of various sub-fields of conduit research. (A) Increase in the number of silk-related papers published over a span of 19 years (2020 is not included). (B) Increase over a span of 20 years of the proportion of treatment papers to control papers. (C) Growth over a span of 20 years of proportion of spider and silkworm silk to total silk papers.

from Analysis 1 indicated a growth in the field of peripheral nerve conduit research, especially in the silk subfields.

Statistical Relevance of Included Papers (Analysis 2)

Analysis 2 focused on establishing relevancy of the papers gathered from Analysis 1 through statistical methods, while also narrowing the pool of papers from Analysis 1. First, we set inclusion criteria that had to be met before a paper would be included in the statistical analysis. The papers had to: 1) have an impact factor; 2) be published after 2000; 3) meet the citation number baseline that was established (details in the methods). The papers were then marked as either ‘Y’, meaning they met the criteria, or ‘N’ meaning they did not meet the criteria. Both pools of papers’ average citations were found, and a chi-squared analysis was done additionally on the ‘Y’ pool of papers.

The average number of citations for ‘Y’ papers was 68.75 ± 76.82 (N = 44), while the average for ‘N’ papers was 48.93 ± 61.02 (N = 44). The mean for ‘Y’ papers was higher than that of ‘N’ papers, suggesting that the ‘Y’ papers may be more relevant, but the standard deviation of ‘Y’ papers was higher, indicating more variability within the sample regarding number of citations. A paired t-test was performed, and we found that this difference in the number of citations between the two samples was not statistically significant (paired t-test value = 0.1839, df = 43, p = 0.078).

The second statistical test we did was a chi-squared analysis of total number of ‘Y’ papers in 5-year increments. All assumptions were met: normality and expected value greater than 5 (EV = 11). The two groups we compared in this test were the expected number of ‘Y’ papers (EV = 11 for all 5- year increments) and the number of observed ‘Y’ papers (the observed values were the total number of collected data points). When we applied this test to the data, we found statistical significance (chi-squared value = 36.545, df = 4, $p < 0.0001$). When visualising the proportion of ‘Y’ papers per year, this suggested that during the 20 year period, the proportion of relevant papers increased dramatically from 0% in the early 2000s to a minimum of 40% since 2017 (**Figure 2**).

Paper Summaries (Analysis 3)

Analysis 3 consisted of two steps (Step 1 and Step 2) that were designed to collect data for the last two research questions. We accomplished Step 1 through reading only the title and abstract of the papers. First, the papers had to meet the inclusion criteria of having the following words in the title or abstract: peripheral nerve and conduit/scaffold/tube. After

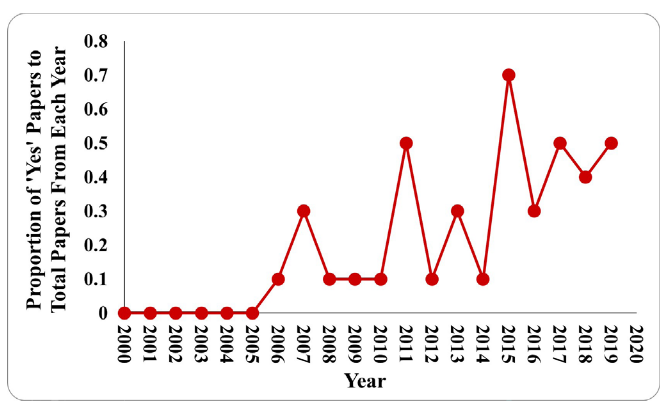

Figure 2: Papers meeting criteria. The proportion of ‘Yes’ papers (papers that meet the specified criteria) to total papers published from each year in order to assess growth in relevancy of papers published.

meeting those criteria, the paper was scored based on the presence of specific words in the title or abstract (words and point allocations specified in methods section).

In Step 1 of Analysis 3, we considered any papers that scored a 2.5 or above for the qualitative analysis in Step 2. The papers had a mean score of 3.1 and average citation count of 98.2. Not all papers were found through the set methodology; additional papers were found through organic search and then scored. Of the five papers analyzed in Step 2, we found three through the Step 1 methodology, and two through organic search. We then carefully read through those five papers and summarized them in **Table 1**.

The most significant finding from the study that Allemeling *et al.* conducted was that spider silk has properties that enhance Schwann cell proliferation and attachment *in vitro* (24). The Ghaznavi *et al.* study was a large *in vivo* study, and it found that silk guides have the same properties as autografts to promote regeneration but do not perform any better than grafts (22). Similarly, the study Radtke *et al.* conducted found that the silk construct led to regeneration similar to the graft but did not provide any further enhancements (21). Huang *et al.* focused on gap length and found that the silk conduit was able to bridge a 13mm gap, something grafts are often unable to do (25). Lastly, the Xue *et al.* study was by far the most unique because it was an *in vivo* study observing the effects of a silk scaffold on an extremely large nerve gap of 30mm (23). However, more interestingly, Xue *et al.* not only examined the physical nerve regeneration, but they also conducted a gait analysis to examine the functional recovery as well (23). The authors, like many of the other studies, found that the scaffold provided no additional benefits as compared to the graft (23). Thorough summaries of each paper are found in **Table 1**.

DISCUSSION

Growth of Field Analysis

In response to our first research question, “How has the field of peripheral nerve regeneration conduit research, and its subfields, grown in the past 20 years?”, we found that silk has been growing in popularity as a biomaterial in nerve conduits, and the published literature substantiates this trend. Through the growth of field review that was done in Analysis 1, it was clear that silk has experienced significant growth over the past 20 years, with the majority of that growth occurring after 2010. Though this growth could have been due to increased awareness, it could also be due to the inception of the Seri Surgical Scaffold Silk in 2009, which was the first commercial biomedical technology using silk in this way, potentially inspiring research into using silk for conduits as well.

We further found that spider silk initially had the fastest growth, but by 2005, silkworm silk had also started to garner interest. And by 2020, silkworm silk was used as frequently as spider silk. This growth of silkworm studies could be due to numerous factors, but the most prevalent ones are the following: primarily, spider silk is harder to harvest in bulk, making silkworm silk more appealing for large scale studies; secondly, spider and silkworm silk do not seem to have any significant property differences, thus making them equally appealing in many regards.

Figure 2 showed that the proportion of ‘yes’ papers increased over the past 20 years, which could indicate that papers meeting the Analysis 3 inclusion criteria were getting more relevant over time.

Limitations

Over the course of implementing the designed methodology, some limitations were noted. It should be considered that Google Scholar may filter out applicable papers, and that such papers may not make it onto the first results page. For Analysis 2, number of citations and impact factor of the journal may not always be an accurate measure of relevancy or quality of a paper. Lastly, Analysis 3 had many papers that passed the score threshold, but were still not applicable, indicating that there was a lack of some more specific exclusion criteria.

Silk’s Potential as a Nerve Conduit

Of the five papers that passed through Step 1 and were subjected to a final analysis in Step 2 in Analysis 3, four of the papers concluded that silk performed the same as traditional nerve grafts and did not provide any enhanced benefits for overall regeneration (21-23, 25). However, Allemeling *et al.* conducted a study in which they were able to show *in vitro* that spider silk specifically enhanced Schwann cell proliferation, indicating that perhaps there are characteristics of silk that have yet to be tested that could provide benefits to the regeneration process and perhaps that these characteristics are specific to spider silk (24).

The most important aspect found was evidence of the two most compelling benefits of silk: biodegradability and biocompatibility (22, 23). In the *in vivo* study done by Ghaznavi *et al.*, macrophage count was recorded and showed that immune response was much higher with the autograft, whereas there was a minimal initial response that subsided to less than 5% with the silk conduit. This indicates that silk does not evoke a toxic response and is less immunogenic than nerve graft (22). In terms of biodegradability, the *in vivo* study done by Xue *et al.* found that after 12 months, most of the silk scaffold had degraded and been absorbed safely by the body, supporting the claim that silk is a naturally degradable material that does not cause any harmful reactions upon degradation (23).

This final analysis also helped to answer our second research question of “What are previous successful and unsuccessful approaches?”. Overall, we did not categorize any of the five papers as having unsuccessful approaches because all studies provided conclusive data. However, there were some approaches that were subjectively more successful than others because of they were more comprehensive or because they highlighted more niche properties of silk that could be particularly significant in future research. This success was quantitatively gauged through our Step 1 scores early in our final analysis. The papers that we classified as comparatively successful are those with the highest Step 1 scores: Radtke *et al.* with a score of 3.5 and Huang *et al.* with a score of 4 (21, 25). The Radtke *et al.* study was especially unique because it was a large *in vivo* study that utilized both a gait study to determine functional recovery and used immunostaining to examine axon regeneration. Huang *et al.* conducted a study that also stood out because it was the first study that concretely showed that there may be advantages of using silk fibers versus grafts regarding efficacy of gap length bridging.

Overall, from this sample of five papers, the consensus was that nerve grafts remain the ‘gold standard’ for nerve repair, but there are still numerous factors that must be considered when choosing between a graft or a silk conduit.

Nerve grafts themselves have many limitations that make a synthetic alternative more appealing. If the graft is coming from a human or even non-human donor, there is the huge risk of rejection by the body, but if the graft is an autograft, there is a limit to the length of gap that could be bridged with such a graft and potentially could harm the part of the body that the graft is coming from (9). Silk conduits can bridge any length of gap, have been proven to be biocompatible, do not require damage to any part of the body, and only require one-time surgery due to natural degradability (17, 20, 21). Thus, silk conduits are very promising and potentially safer options than traditional grafts in certain situations.

Gaps and Future Studies

To address our final research question, "What are areas for

Paper	Summary
Use of spider silk fibres as an innovative material in a biocompatible artificial nerve conduit (24). Citations: 165 Step 1 Score: 2.5	Allemeling et al. conducted an *in vitro* study using cultivated adult human Schwann cells (SC) and spider silk fibers. SC were dropped onto the spider silk filaments and then observed periodically for attachment. A control of polydioxanone monofilaments (PDS) was also conducted. It was found that after as few as 15 minutes, there was proper attachment of the SC to the silk whereas it took 120 minutes for attachment to PDS. After 24 hours of incubation, the spider silk fibers were completely ensheathed in viable and properly attached SC, whereas the PDS was only sparsely covered and not all cells were viable. Since this *in vitro* experiment showed the favorable properties of the silk, the nerve construct was then built using the spider silk fibers and acellularized veins. After 1-week of *in vitro* cultivation, the nerve conduits showed that the SC were vital, enclosed the silk fibers completely, and were oriented in longitudinal, nerve-like bundles along the fibers. It was concluded that spider silk has favorable properties that enhance SC proliferation and attachment, but further *in vivo* studies are needed.
Silk Fibroin Conduits: A Cellular and Functional Assessment of Peripheral Nerve Repair (22). Citations: 77 Step 1 Score: 2.5 **found through organic search*	This study was an *in vivo* study that utilized porous silk tubes and 45 male Lewis rats. The rats were split into 4 treatment groups: autograft, silk nerve guide, collagen nerve guide, and control. Two main parameters were measured: mean fiber counts/fiber density and macrophage infiltration. There was no difference seen in the percentage of nerve tissue, but it was found that fiber density and myelin maturity was better with the silk guide. After week 1, the macrophage count was significantly higher in the autograft group than in the collagen and silk guide groups. Macrophages were detected in response to the silk guide at week 1, but decreased to less than 5%, most likely due to the degradation of the silk. The study concluded that silk guides do not enhance regeneration but have the same properties as autografts to promote successful nerve repair.
Spider Silk Constructs Enhance Axonal Regeneration Remyelination in Long Nerve Defects in Sheep (21). Citations: 98 Step 1 Score: 3.5	This paper detailed an *in vivo* study involving 24 sheep and *Nephila* (spider) silk. The sheep were divided into 2 treatment groups: autologous transplant group and silk construct group. A gait analysis was performed, as well as myelinated axon counts, immunostaining, and electrophysiological recordings. The gait analysis showed that the best functional recovery occurred in the group with the silk guide. Immunostaining showed that in both the autologous and construct groups, axons had regenerated to bridge the entire length of the defect. However, the staining showed that Schwann cell migration was deficient in the autologous graft, but they fully ensheathed the axons in the construct. There was no statistically significant difference in the myelinated axons counts or the electrophysiological recordings between the two groups. It was concluded that this construct led to regeneration similar to the autologous graft.
Regenerative potential of silk conduits in repair of peripheral nerve injury in adult rats (25). Citations: 119 Step 1 Score: 4	This study tested variations of the *Spidrex* fibers *in vitro* for their ability to support neurite growth over an 8 mm nerve gap. Four variations were used: PN0, PN100, PN200, and PN300 (PN refers to the diameter/density of the silk fibers). The PN200 group achieved the best axon regeneration compared to all the other groups and was chosen for more extensive analysis against an autologous group. It was concluded that though the PN200 group achieved faster and better recovery than the PN0 group, there was no statistically significant difference between the PN200 and autologous groups. However, the PN200 were also used in a larger 13mm nerve gap, and it was shown that the gap was effectively bridged, something that is more difficult to do with autologous grafts.
Electrospun silk fibroin-based neural scaffold for bridging a long sciatic nerve gap in dogs (23). Citations: 32 Step 1 Score: 3 **found through organic search*	An *in vivo* study was done using 27 male adult Beagle dogs with a 30 mm long sciatic nerve gap. The silk-fibroin based neural scaffold was made using electrospun *Bombyx mori* (silkworm) silk. The dogs were divided into three treatment groups: SF-scaffold group, autograft group, and a non-treatment group. A gait analysis and nerve functional evaluations were performed 12 months after surgery. It was found that after the 12 months, the majority of the SF scaffold had degraded and had been replaced by a nerve like tissue, bridging the 30 mm gap. Dogs from the scaffold group showed symmetric gait, whereas dogs from the non-grafted group walked with a limp along with other complications. In the nerve functional evaluations, the non-grafted dogs had the worst performance, but there were no statistically significant differences observed between the SF-scaffold group and the autograft group. It was concluded that since no significant differences were found, the scaffold could serve as an effective artificial intervention, but does not provide any increased benefits compared to the autograft.

Table 1: Summary table of the five papers that met the Step 2 inclusion criteria and were deemed relevant enough to be fully analyzed.

future studies?", we reanalyzed all of our collected data and papers in order to find research gaps. Two major gaps were identified: growth rate and length of nerve gap. First, growth rate itself did not seem to have been measured and calculated for each study, but rather just indicators of growth and recovery. It would be useful to have a growth rate to compare to the average self-healing rate of 1 mm/day and see how that rate changes with gap length, type of graft, and silk conduit (4). Secondly, longer nerve gaps that have been shown or hypothesized to be effectively repaired using grafts need to be used in order to determine the efficacy of these conduits in a setting where traditional treatments are not a viable option.

In general, there needs to be more long term, *in vivo* studies in order to properly gauge functional recovery of the nerve and to observe biodegradability of the conduit. Additionally, no paper has directly compared properties of spider silk and silkworm silk in both an *in vitro* and *in vivo* setting. We hypothesize that the main difference between the two silks would be mechanical due to the makeup of spider silk (18), but there is a need for a comprehensive study comparing the two in order to determine if there is a significant difference or not.

The next step for many of these studies would be to either move on to an *in vivo* implementation on non-human animals or a clinical trial to determine feasibility in humans. According to clinicaltrials.gov, there is one active clinical trial right now that is specifically using a silk-based conduit known as SilkBridge to treat peripheral nerve injury in the hand (26). This study is the first step in implementing such conduits in human patients.

Our findings indicate an increase in overall interest and research into biomimetic silk-based conduits over the past 20 years, and the general upward trends shown in **Figure 1** indicate that this field of research will continue to grow. Silk conduits have many promising characteristics, but the literature suggests that nerve grafts currently remain the best treatment. However, further studies, especially human *in vivo* ones, are required to properly ascertain whether silk-based conduits truly provide any additional benefits and whether they can be viable alternatives to grafts in the future.

MATERIALS AND METHODS

Overall Methodology

The methodology devised is a comprehensive 3-analysis approach comprising both inclusion and exclusion criteria. The majority of this approach is bibliometric, meaning that raw data was collected, and was then subjected to various statistical tests, allowing for proper analysis. Though it is a bibliometric analysis, this approach is designed to obtain both quantitative and qualitative data in order to effectively evaluate the relevancy and quality of the papers. We collected all data in August 2020.

The objective of the first analysis was to establish an understanding of how the field of peripheral nerve regeneration conduit research has grown in the past 20 years since 2000. We created a control search phase and a list of treatments to be typed into Google Scholar. The control phrase was: "peripheral nerve" repair conduit. The structure for the addition of treatments was: "peripheral nerve" repair conduit [treatment]. The treatments used were vein, artery, hydrogel, polymer, ligament, collagen, silk, "spider silk," and "silkworm silk." For each treatment, including the control, the search parameters were specified to only show data for one year at a time; the custom time range, for example, showed 2000-2000 in order to gain data for only the year 2000. In the end, there were 200 total data points (10 for each treatment per year). The data points that we collected were the total number of papers published during that year, located at the top of the first results page, which served as a basis to allow analysis of growth.

Analysis 2-3 were similarly repeated, but we only used the search phrase "peripheral nerve" repair conduit silk. The first ten papers shown on the first results pages were subjected to each following analysis.

The main objective of Analysis 2 was to use basic statistics to narrow the pool of papers. The first two criteria were that the paper had to have been published in a journal that has a Clavariate Analytics Impact Factor (IF) and it must have been published in the year 2000 or after. The third criterion was the number of citations referencing that paper. We used the number of total citations of each paper as a crude proxy for impact and importance of the paper. If the paper was published between 2000-2005, a minimum of 35 citations was required; if published between 2006-2010, a minimum of 25 citations; if published between 2010-2013, a minimum of 10 citations; if published between 2014-2017, a minimum of 5 citations; if published between 2018-2020, there were no citations required. The final criterion required the reading of the title of the paper and the skimming of the abstract, if necessary: the title or abstract of the paper had to have contained the word "silk." If any paper did not meet all four criteria, we eliminated it as a prospective paper for further analysis.

Analysis 3 involved two iterations: Step 1 and Step 2. In Step 1, if the paper was a literature review, we eliminated it. The following words must have been in the title or abstract, or the paper was eliminated: peripheral nerve and conduit/scaffold/tube. After meeting that primary inclusion criteria, the papers underwent an additional objective scoring. This scoring system was developed because the mandatory inclusion criteria by itself was not sufficient to assess relevancy of the paper. We chose the words carefully after preliminary research and analyses of core papers. Specific words were assigned points based on their significance, and for each word that the title or abstract had, the paper would receive that many points. If the study was *in vivo* (0.5 point), *in vitro* (0.5 point), or both (1 point). Regarding identification of silk: spider silk (1 point), silkworm silk (1 point), or reconstituted silk/silk fibroin (0.5 point). Lastly, if gap length was listed (1 point) or if Schwann cells/Schwann cell migration was mentioned (1 point). By considering the mandatory inclusion criteria coupled with the number of points the paper scored (the score had to be at least 2.5), we determined whether the paper would be suitable to move to Step 2.

The goal of Step 2 was to evaluate the paper for quality and comprehensiveness. Once a paper moved on to Step 2, we read it fully and assessed the following criteria: whether it included a clear explanation of how the neuronal growth was measured (immunohistochemistry, standard of measuring, and/or analysis of pictures taken); whether the results were explicitly stated; whether the results showed something significant such as decreased regeneration, no rejection of conduit, etc.; and whether there was a comparison to other silk conduits and/or currently available treatments. If a paper met 3/4 of those criteria, we deemed it to be a well-written paper.

Data Analysis Methodology

The indicator of growth used in this analysis was the number of papers published for each treatment each year and the proportion of 'treatment' papers to control or silk papers for each year. Control papers were the group of papers that used only the control search phrase with no additional treatment. The total number of control and total number of silk papers were graphed in order to evaluate general growth over the past 20 years. To gauge the growth of silk specifically, we graphed two proportions: the proportion of silkworm papers to total silk papers and the proportion of spider silk papers to total silk papers. All other treatment groups were graphed as a proportion of total control papers in order to assess growth of each of those niche fields.

Analysis 2 allowed for the collection of data regarding number of citations of papers published each year, impact factor, and presence of silk in title or abstract, all three of which were criteria that had to be met to advance to Analysis 3. Each paper that passed the inclusion criteria was labelled with 'Y' and each paper that did not meet the criteria was labelled with 'N.' Normality for the statistical tests was tested through a histogram which, if it had an approximately normal bell shape, would satisfy the condition.

The first two calculations done were averages and standard deviations. The first statistical test we performed was a two-tailed paired t-test to compare the number of citations of 'Y' papers and 'N' papers. The two samples used for all three of these calculations were the number of citations for each 'Y' paper and the number of citations for each 'N' paper. In order to account for Type 1 errors, we developed a randomization method to identify which 'N' papers to use. For each year, there had to be the same numbers of 'Y' papers to 'N' papers. We used, a random number generator set to pick a number from 1-10 to create a corresponding and randomized sample of 'N' papers. Within each year, all 10 papers were labelled 1-10. Separately, each 'Y' paper was listed chronologically and with number of citations. We then used the random number generator to identify which remaining 'N' paper and its number of citations would be paired to a specific 'Y' paper within the same year. The second statistical test done was a chi-squared analysis of total number of 'Y' papers in 5-year increments (not including 2020). All statistical analyses were done using Microsoft Excel.

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