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Regenerative Engineering and Bionic Limbs

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Abstract

Amputations of the upper extremity are severely debilitating, current treatments support very basic limb movement, and patients undergo extensive physiotherapy and psychological counselling. There is no prosthesis that allows the amputees near-normal function. With increasing number of amputees due to injuries sustained in accidents, natural calamities and international conflicts, there is a growing requirement for novel strategies and new discoveries. Advances have been made in technological, material and in prosthesis integration where researchers are now exploring artificial prosthesis that integrate with the residual tissues and function based on signal impulses received from the residual nerves. Efforts are focused on challenging experts in different disciplines to integrate ideas and technologies to allow for the regeneration of injured tissues, recording on tissue signals and feed-back to facilitate responsive movements and gradations of muscle force. A fully functional replacement and regenerative or integrated prosthesis will rely on interface of biological process with robotic systems to allow individual control of movement such as at the elbow, forearm, digits and thumb in the upper extremity. Regenerative engineering focused on the regeneration of complex tissue and organ systems will be realized by the cross-fertilization of advances over the past thirty years in the fields of tissue engineering, nanotechnology, stem cell science, and developmental biology. The convergence of toolboxes created within each discipline will allow interdisciplinary teams from engineering, science, and medicine to realize new strategies, mergers of disparate technologies, such as biophysics, smart bionics, and the healing power of the mind. Tackling the clinical challenges, interfacing the biological process with bionic

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technologies, engineering biological control of the electronic systems, and feed-back will be the important goals in regenerative engineering over the next two decades.

Keywords

Bionic; electrical stimulation; regenerative engineering; muscle; nerve; prosthetic

1 Introduction

There are approximately 1.25 million amputees living in the United States, with 135,000 new amputations performed each year; the peak age for amputations is between 41 and 70 years of age, with 75% of amputations occurring in those over 65; 70% of amputations are due to disease, 22% to trauma; 4%, to congenital malformations, and 4% to tumors [1–7]. The recent conflicts occurring world over have resulted in a significant increase in younger active individuals undergoing extremity amputations. Government agencies and private organizations have increased funding into research and product development programs that have potential to minimize loss of life and loss of limbs in conflicts. Most amputations are the result of trauma, and the US Department of Defense has committed more than \$150 million into next-generation prosthetic development program known as ‘Revolutionizing Prosthetics’ [8]. For most upper-extremity amputees, the currently available upper limb prosthetic systems are inadequate and only allow for a minimal and poor quality of movement. The current artificial limbs are very difficult for an amputee to control efficiently largely due to lack of sensory feedback. In response to the increasing need for a more functional prosthetic limb system, in recent years there is increased funding available and several active programs have been initiated [9–18].

Upper limb amputation is a life-altering disability that interferes with basic daily activities including feeding, personal hygiene, and changing clothes [19, 20]. The key challenge for amputees is learning how to control their movements using their prosthetic limb. Amputation results in significant functional and cosmetic changes which cause the individuals to be affected psychologically. Clinical experiences have demonstrated that the prosthesis will be eventually rejected by the patients if it fails to fulfill their expectations regarding cosmetic appearance, function and subjective considerations. Early prosthesis options included passive devices that were esthetic and shaped to the contours of the amputated limb. They passive devices have no functionality, and general is made of light-weight materials that required minimal harness and little upkeep. Other iterations of passive prostheses included harnesses and cables, and at times rudimentary myoelectric components that performed basic functions such as open and close. Amputees underwent extensive occupational therapy to learn how to perform required tasks, and psychotherapy to overcome emotional problems, depression, and helplessness.

The development of new conductive biomaterials (metals and polymers) and technologies has realized novel flexible electronics based systems that are biocompatible, provide mechanical tissue support, and more specifically incorporate sensors and are able to deliver electrical stimuli to affect controlled limb motion. Recent prosthesis’s under design incorporate such novel technologies allowing sufficient tissue regeneration and sensors to

facilitate deliberate and controlled complex movements and tactile feedback of the mechanical joints in response to stimuli from residual muscle groups and nerve bundles. Early studies have demonstrated correlation between increased vasculogenesis with electrical stimulation, and implantable electrodes form cell contacts enabling the recording and stimulation of nerves. The combination of electrical stimuli and prosthetic interfaces using implantable metal electrodes and sensors may indicate an alternative strategy to modulate tissue regeneration and repair, and transmit and record stimuli from residual muscle and nerve groups. Furthermore, in the human body, various metallic ions are involved in cell signaling pathways as cofactors of enzymes and are essential to cellular mechanisms [21–23]. Metals ions have a significant role in a wide range of pathological conditions such as cancer, central nervous system disorders, infectious diseases, and endocrine disorders. Properties such as hydrolytic and redox activity, electrophilicity, valency, geometry, magnetic effect, spectroscopy, and radiochemical properties of metal ions influence interactions modulating cell metabolism, and biological functions (binding to enzymes and nucleic acids, activating ion channels). Regenerative strategies involving metal ions offer a novel opportunity by incorporation into engineered biomaterial scaffolds, which additionally provide high flexibility to the implant devices.

2 Prosthetic Systems

Today, the most commonly used prosthesis is body-powered; a largely mechanical device. For instance, upper-limb prosthetic devices capture the remaining shoulder motion with a harness, and transfer this movement through a cable to operate the hand; wrist or elbow joints and typically only one joint can be operated at a time (Fig. 1a) [24]. Users must lock the joints they wish to keep stationary thereby allowing them to switch between various basic functions. The harnesses required for functionality and suspension limits the range of motion of the prosthesis, and range of motion around the individual that can be utilized without restricting movement of the other limbs. Strenuous and large-movement activities are limited and necessitate the use of body motion. Amputees have been largely observed relying on use of the intact limb for such functions. In addition, long-term use of a body-powered prosthesis has been correlated to debilitating shoulder issues, nerve entrapment or pinching and anterior muscle imbalances [25, 26]. The second most common prosthesis is motorized relying on surface electromyogram (EMG) signals from the residual muscles sites (Fig. 1). Control of movement is achieved using electrical signal recordings of muscle contraction from either two independent muscles or by differentiating weak and strong contractions of a single muscle, and converting those signals into those that influence electrical motors [27]. The amplitude of the EMG signal is used to operate various functions of the prosthesis. For example, the bicep and triceps muscles are used by an above-elbow amputee to control the elbow, wrist and hand. The contraction of bicep and triceps muscles will control elbow flexion and extension or terminal device closure and opening. A below-elbow amputee will contract wrist flexor and extensor muscles to control terminal device closure and opening. Some of the myoelectric prosthesis use force-sensors that can interpret pressure applied by the finger grasp in a linear manner. Proper installation and calibration of such input-output systems can significantly contribute to the success of the prosthesis especially in providing controlled functionality or calibrated limb movement. A third option

combines the benefits of body-powered prosthesis with EMG signals to form hybrid prosthesis, such as one having a mechanical elbow combined with a myoelectric terminal device [19, 24]. These hybrid devices allow the user to simultaneous control multiple movements such as elbow and terminal device. Such hybrid devices are essential for people with the most severe upper-limb disability which is transhumeral or shoulder disarticulation amputations. Below-elbow amputees only need to control hand and wrist motion, the transhumeral amputee must use shoulder muscles to control hand, wrist, and elbow motion. This is even more problematic because there are few native muscles to power the prosthetic limb, and there is no humerus to position the terminal device in space. The most prevalent type is a body-powered elbow and electrical connections to move wrists and for finger grasp.

Often due to exaggeration in media and films, many amputees have high expectations for the degree of accomplishments that upper-limb prosthesis can deliver, with patients in many cases expecting futuristic devices [28, 29]. Though the prosthetics are technically very sophisticated they are far less advanced than expected by the amputees. The choice of treatment is unique to every individual and the rehabilitation team must tailor each to the abilities and preferences of the patient. Major limitations in controlling movement using myoelectric signals is the difficulty in achieving reliable recordings of EMG signals and the lack of sufficient sensory inputs for the various actions expected to be performed with the prosthetic limb. More proximal amputations suffer from significant loss of input sources making it harder for the patients to produce isolated EMG signals and repeatable contractions. Additionally, the changing conditions of the skin such as sweat and loss of sensor adhesiveness makes it challenging and unreliable. The limited amount of input information results in reduced function and control, which contributes to patient frustration and prosthesis abandonment. New technologies developed in the last decade such as implantable EMG electrodes, EMG pattern recognition, and targeted re-innervation, may address some of the problems inherent in traditional myoelectric control.

3 New Treatment Modalities for Limb Amputation

Over the last decade, new treatment options have been developed namely, composite tissue transplantation and targeted muscle re-innervation (TMR) [30–32]. These approaches vastly differ from the strategies used till now and have the potential to restore most functionality if not all depending on the level of amputation, length and risk of the procedure, associated costs, and recovery. Transplantation has the potential to restore both the function and appearance of a human limb providing immeasurable psychological benefits. However, the clinical procedure is by-itself extremely challenging requiring multiple surgical teams, and patient requires lifelong multi-drug immunosuppressive treatment [33–37]. Immunosuppressant drugs are associated with secondary medical complications leading to severe life-threatening side effects. The clinical and monetary requirements of performing a successful limb transplantation surgery prohibit widespread application of this treatment strategy in any part of the world, including the most developed countries. About 50 hand transplantation procedures have been completed since 2009, with majority being at the level of the wrist and a significant number at the mid or distal forearm. Graft survival has been excellent at 95.6%. Hand transplantation procedures involves significant recovery time to

establish osteointegration, tendon healing, and finally muscle re-innervation prior to beginning active rehabilitation.

The technique of targeted motor re-innervation first reported in 2004 has become one of the most important advancement in prosthetics [31, 38–40]. This innovative surgical procedure reroutes signals from nerves severed during amputation to intact muscles, allowing control of prosthetic devices by merely thinking about the action (Fig. 2a) [40]. Amputated nerves are transferred to spare or residual muscles in the residual limb and they grow into the muscle to provide additional control signals for prosthetic limb operation. The large transferred nerves contain both motor and sensory axons. This allows patients to simultaneously control multiple functions in their prosthesis in an easier and more natural manner. Surgeons first denervate regions of residual or other muscles and then transfer the residual peripheral nerve endings to them. The nerves re-innervate these muscles, and surface EMG signals from the newly re-innervated muscle serve as additional control signals for an externally powered prosthesis. For instance, transfer of the median nerve to the medial head of the biceps brachii causes muscle contraction generating an EMG signal (Fig. 2b) [38]. Using myoelectric technology, the terminal device translates that EMG signal into device closure. The patient has to think of performing a motion (e.g. hand movement and finger closure), and the target muscle will contract and EMG signals are translated to an action performed by the device. TMR amplifies the information present in the residual nerves by contraction of the re-innervated muscle and allows for controlled movement of the prosthetic limb. This novel treatment can overcome limitations that are evident with current motorized prostheses, such as being able to operate one motion at a time. In some patients, the sensory nerves present in the vicinity of the transferred motor nerve can be coapted to the median or ulnar nerve for targeted sensory re-innervation. The sensory nerves will regenerate through the muscle making terminal connection to the denervated skin that is overlying the transfer site [41, 42]. This approach results in a sensation of the patient's hand being touched when stimulating the re-innervated skin. The patient senses the phantom limb [39, 43]. Patients are able to discriminate between variations in applied force comparable to that experienced on the uninjured skin and limb. This feature not only improves the utility of the prosthetic device, but allows the patient to integrate the prosthesis as an extension of them and significantly improves their perceived image. The restoration of neural communication signals through the peripheral nerve branches to the nerve trucks enables one to feel pressure and sensations such as vibration, heat, cold and pain as if applied to the missing limb.

Following amputation, the control signals of the lost limb is present in the residual peripheral nerves of the amputated nerve branches or trunk. A novel innovative approach utilizes electrodes connected directly to the residual nerves to control the artificial limb [44, 45]. These conductive materials are permanently inserted and record from several neuronal sources enabling precise movement of the prosthetic upper-limb [46–48]. For instance, electrodes connected to wrist flexion motor axons could be used to control a powered wrist flexor, or motor axons of the thenar muscles could be used to operate a powered thumb. Similarly, the afferent fibers might be stimulated to provide sensory feedback of touch, temperature, and position. These implanted materials are not subject to the environmental factors that affect surface recordings and could therefore provide more stable and consistent

control minimizing the challenges associated with surface EMG recordings. Implantable conductive materials or electrodes have been successfully demonstrated in short-term studies and various challenges need to be overcome for long-term success. The long-term requirements include complex implantable transmitter-receiver systems to avoid use of percutaneous wires which tend to become infected. Additionally, motor nerves may atrophy due to the lack of contractile forces of the muscle tissue.

4 Bionic Arm

Jesse Sullivan, a bilateral upper-limb amputee became the first person to undergo experimental TMR procedure after losing his arms in an electrical accident. The nerves in his amputated upper-limbs were transferred to spare muscle and skin in the left side of his chest, and this procedure enabled him to independently move different joints of his prosthetic arm intuitively. These impulses are sensed, via surface electrodes placed over the pectoral muscle and transmitted to the mechanical arm causing movement. He was dubbed the “Bionic Man”. He could feel the sensation of someone touching his missing arm when they touched the skin over his TMR muscles. Over the last decade, several other patients have undergone TMR procedures which typically involve 2–6 nerve transfers and takes about 4–6 months to achieve adequate re-innervation, following which the amputee is fitted with a myoelectric prosthesis. However in subjects having undergone shoulder or transhumeral amputations there is insufficient usable skin and muscle due to damage, scar tissue formation and inadequate muscle volume and is challenging to obtain sufficient input signals for individual control of prosthesis functions.

The next generation of prosthetic devices will rely of inputs received directly from the nervous system including the central and peripheral nerves. Nerve signals can be detected by metal electrodes placed inside or adjacent to the nerve bundle (Figs.3 and 4) [44, 49]. These signals are significantly smaller in amplitude than the EMG signals from muscle contractions, however they are highly reliable and the availability of numerous input sources, and sensory and perceptive signals providing stimulatory feedback make this a very attractive solution. Researchers have demonstrated that motor neuron signals of the amputated limb remain viable and have used both extra-neural and intra-neural electrodes as input sources and for stimulatory feedback [44, 50]. This novel strategy has driven the need for saving vascularized innervated muscles for the implantation of myoelectric electrodes. With the increasing degrees of freedom in the latest prosthesis, one requires several interfaces with the nerve trunk and individual nerve branches to allow for numerous input sources that are responsible for movements such as flexion of the digits, extension, wrist motion, movement of thumbs and all joints of the upper-extremity such as wrist, elbow and forearm. Implantable electrodes made of novel conductive materials have been used to tap into the visual cortex (central nervous system) to restore eyesight using a bionic visual prosthesis and also to aid hearing [51, 52]. These experimental studies demonstrated reliable signal recording from peripheral nerves using novel implantable electrodes. A neural electrode placed surrounding the nerve is able to record and also stimulate the nerve (Fig. 3) [53–55]. Such conductive electrodes include the nerve cuff and the flat-interface nerve electrodes. Nerve cuff electrodes are effective for long-term use and currently used in the Otto Bock’s ActiGait® system to correct foot drop [56]. The nerve cuff records and

stimulates simultaneously several nerves that are present in the nerve trunk. The flat-interface electrodes involve flattening the nerve bundles to establish contact between an array of electrodes and individual nerves allowing for multiple signal outputs and specific stimulatory inputs. Intra-neural electrodes are placed through the nerve enabling the stimulation and recording of individual and small nerve bundles. These electrodes penetrate the nerve bundles thereby allowing for greater specificity than extra-neural electrodes (Fig. 4) [57–59].

Researchers have used thin metal electrodes composed of biocompatible materials placed parallel to the nerve fibers and within the individual nerve fibers. These longitudinal intrafascicular electrodes (LIFEs) have facilitated reliable recording and direct nerve stimulation in approved human clinical trials where they were placed into the median nerve of several amputees [60]. Many of the patients were able to control grip and the joint movement of their prosthetic hand in proportion to the neuronal signal rate measured by the implanted electrodes. In addition, these electrodes enable them to distinguish between gradations of force and joint angles which were conveyed by various stimulation intensities of the sensory nerve. Researchers at the University of Utah developed multi-electrode arrays containing dozens of electrodes arranged on a rigid base and placed them into the nerve penetrating through it (Fig. 4h) [58, 61–63]. This intra-neural placement improved selectivity and minimized interference and cross excitation. The Utah Slant Array as it is known has been evaluated in large animals and in limited human subjects where they have been implanted up to one month during which they could successfully perceive sensation and generate action-input signals corresponding to hand tasks performed by the amputated limb. No adverse reactions were reported in the surrounding muscle and nerve stump. In some subjects, nerve proliferation in response to electrode signaling was observed. The safety and stability of the interfaces formed between the metal electrode material and the nerve is a critical challenge.

Major challenges in the successful application of implantable electrode systems involve the safety and stability of the electrode-nerve fiber interface. The materials used in fabrication of the electrode must meet requirements of electrical stimulation and reliable recording of nerve triggers. In general metals having high impedance are avoided for neural recording and stimulation. The contact formed between the electrode and cell and the array geometry influences the recording from and the stimulation of the nerves. Electrodes of smaller cross-sectional area and rougher surface area increase the contact coupling between cells and electrodes [64–66]. Nanotexturing technique increases the surface roughness by many orders of magnitude and roughness in range of nanometers to micrometers is reported to enhance electrochemical conduction. The surface roughness and porosity of the electrode serves as an excellent substrate to support neuronal growth and neurite extension.

5 Regenerative Engineering and Limb Replacement

Despite the many important advancements at improving the quality of life of amputees, none of these lead to the total regeneration of an amputated body part. Developing a new engineered strategy that could regenerate a lost body part or integrate with advanced prosthesis is the ideal solution to this problem for improving the quality of life for these

patients considerably [3, 10, 67, 68]. Over the past 25 years, advances have been made in biomaterials-based approaches to repair organ systems. In the past decade, three areas of technology have emerged, namely nanotechnology and advances in materials science, secondly, stem cell science and thirdly, better understanding of developmental biology mechanisms, and the role of the blastema in regeneration which has strengthened and furthered our efforts in wound repair and regeneration. We believe the future of tissue healing (regeneration and repair) will be facilitated by the “regenerative engineering.” Each of these scientific advances has matured to the extent that they are now regarded as tools rather than simply concepts and ideas. The challenge in prosthetic limb replacements is to incorporate tissue regeneration and long-term integration with prosthesis and provide sensory feedback so that patients perceive the device as being an extension of their body [69–72]. Currently, commercially available prosthesis is able to provide some sensory feedback such as the sensation of touch, heat and cold. They however are severely lacking in tactile and force feedback. Regenerative engineering will harness and expand the technological tools by ‘convergence’ of interdisciplinary teams from the fields of engineering, science, and medicine which include scientists, engineers, physicists, and clinicians who have integrated training that spans these disciplines [10, 13, 14, 17, 73–77]. The development of new conductive biomaterials and technologies has led to the design of flexible electronics based systems that simultaneously serve three distinct functions: to mechanically support tissues such as muscles and nerves, locally deliver biochemical cues and finally, localize or modulate electrical stimulation [78].

The role of external forces in normal tissue development and tissue remodeling is well studied. These external forces such as electrical stimulation, ultrasound, mechanical loading, and electromagnetic fields have been used to stimulate damaged tissue and accelerate the healing process. For instance, electrical stimulation (ES) has been shown to enhance cell multiplication in connective tissue and in formation of new collagen in injured tissues [79–81]. Severed dog tendons have been shown to return to 92% of normal strength in 8 weeks when subjected to 20 micro amp direct current using implantable electrodes [82, 83]. Electrical stimulation was correlated with increase in new blood vessel capillaries and fibroblast number at the wound site, and followed by increased collagen synthesis. In other studies, expression of vascular endothelial growth factor (VEGF) protein increased in rabbit skeletal muscles after 3 days and up to 8 weeks following electrical stimulation [84]. The release of nitric oxide-like humoral agents that are critical modulators of angiogenesis are increased following ES thereby increasing blood flow to the targeted local tissues [85, 86]. Chronic ES induced vessel proliferation (increased vessel density) and increased VEGF protein in the stimulated skeletal muscle (tibialis anterior and extensor digitorum longus muscles) of Sprague-Dawley rats has been reported [87]. Strategies for bio-integrated electronics must overcome challenges associated with the mismatch between the hard, planar surfaces of semiconductor wafers and the soft, curvilinear tissues of biological systems [88]. These differences in mechanics and form result in low-fidelity coupling at the interface and limited long-term tissue integration. Silk protein produced by silkworms and spiders has been fabricated into a variety of biomaterials, such as gels, sponges and films, for medical applications [89–91]. Silk polymer is bioresorbable and can be fabricated with programmable rate of dissolution. By controlling polymer properties such as degree of

hydration and the degree of crystallinity of silk biopolymer, researches have tuned the dissolution rates and phase-transition dynamics on exposure to water or enzymes. This has enabled the fabrication of flexible electrodes that conform to curvilinear surfaces and thus form close contacts with biological tissues. Ultrathin electronics supported on bioresorbable substrates of silk have been fabricated, and when placed on living tissue causes the silk to dissolve and resorb, initiating spontaneous, and tissue conforming electrode-tissue interfaces. Researchers have reported the fabrication of silicon based electronics in the form of nano-membranes on biocompatible silk substrates [91, 92]. Silicon circuits were printed on ultrathin sheets of polyimide (PI) and then transfer printed onto water-soluble and resorbable silk membranes. The flexible and biocompatible nature of the devices allows for insertion into the body and conforms to the tissue surface (Fig. 5). Simple fabrication techniques such as single step transfers of metal micro patterns to silk films under ambient conditions is very appealing because it allows the simultaneous fabrication of microstructures over large areas of flexible silk films with high precision. Others have demonstrated that functionalized silk films doped with gold nanoparticles (Au-NP) that are light-activated heating elements which when interfaced with thermo-electronic components can wirelessly power micro devices [93]. Au-NP doped silk films were interfaced to thermoelectric chips by solution casting the polymer solution onto the active area of the chip. By using lasers to match the absorption peak of the Au-NPs, a temperature increase in the silk layer was induced which generated 20 mW of power. The development of silk based flexible electronics offers significant opportunities to monitor electroactive functions within biological tissues and enable stimulatory feedback.

The skeletal muscle tissue is a highly organized structure composed of longitudinally aligned, multinucleated muscle fibers formed together by connective tissue to form a densely packed structure [94, 95]. A muscle tension gradient is established by the nervous system, where the motor-neurons that innervate the skeletal muscle cells controls the number of muscle fibers that are contracting, thereby enabling a wide range of motions by the precise spatiotemporal activation of the individual muscle units. Some of the characteristic features of native skeletal muscle tissues have been replicated in vitro. Researchers have demonstrated that genetically modified muscle cells encoding a light-activated protein can be made to contract on exposure to blue light [96]. Murine skeletal muscle myoblasts were genetically modified and suspended in a hydrogel mixture composed of collagen and matrigel (Fig. 6) [97]. Following gelation at 37 °C, the cells were differentiated into thick (20 µm) functional myotubes which were optically actuated by light pulses. When the myotubes were probed with a LED, the cells in the differentiated tissue contracted in unison, resulting in an observable change in tissue size, and then returned back to their initial position. A single myofiber or several fibers could be probed selectively based on the diameter of the light beam and with a single, short pulse. The observed contraction and relaxation behavior of these in vitro microscale muscle tissues was reported to be qualitatively similar to cm-sized native muscle. The ability to selectively stimulate various parts of the muscle and introduce a muscle tension gradient has the potential to control contractile direction and force. Tissue engineered actuators will enable complex regenerative structures interface the biological process with robotic systems and in addition exert control over the mode of actuation.

Similarly, both, low dose ultrasound (LUS) and high dose ultrasound (HUS) has been commonly used for physical therapy, with such treatment methodologies being very cost-effective. Wound breaking strength (WBS) which refers to tensile strength of the wounded tissue and incisions, is significantly increased on the application of HUS (1.5 W/cm², continuous mode, 1 MHz, 5 minutes, 1 week) [98]. The tissue remodeling phase following any injury is focused on redeveloping the tissue functional properties and improving integration. In contrast, LUS (0.5 W/cm², pulsed mode, 20% duty cycle, 1 MHz, 5 minutes, 2 or more weeks) has been shown to increase collagen deposition with marginal improvement in WBS [98, 99]. Low-intensity ultrasound has been shown to regenerate the peripheral nerve following neurotomy, with Schwann cells having a predominant regenerative role following stimulation [100]. The stronger cellular activity of Schwann cells led to the development of numerous thick nerve fibers promoted by the accelerated recovery of the myelin sheaths. Similar trends have been noted using cell-delivery conduits delivering Schwann cells and ultrasound [101]. Non-union tibial shaft fractures (4 months) in adult patients showed significant reduction in bone gap following application of low intensity pulsed ultrasound for 20 minute duration for a period of 16 weeks (1.5 MHz frequency, 1 kHz repetition rate, 200 μ s pulse duration, 30 mW·cm⁻² spatial intensity) [102]. Electromagnetic field application has been investigated as a treatment modality for various pathologies including diabetes, fracture repair, ligament and cartilaginous injury, and for myocardial and cerebral ischemia [103]. Similar to electrical stimulation, electromagnetic field induces extracellular matrix synthesis, cell proliferation and migration. Using a 50 Hz, 20 mT generator connected to a coil with 50 cm in length and 23 cm in diameter, Patino et al. [103] showed significant decrease in wound size and beneficial stimulation in the wound healing process, when pulsed electromagnetic stimulation was applied twice a day for 35 min in Wistar rats. In clinical trials involving long-bone fracture patients, who presented with delayed union, the early application of pulsed electromagnetic field yielded a higher rate of union than those in the control group after the first three months of treatment [104]. In addition to the significantly increased rate of union, the patients benefitted from an overall reduced suffering time.

A wide variety of metal ions such as cobalt, copper, gallium, iron, manganese, silver, strontium, vanadium and zinc are essential cofactors of enzymes, and are able to regulate specific metabolic processes [21, 23]. This has driven the need for localized, and controlled and sustained release of metallic ions for therapeutic applications which modulate cellular functions, cell metabolism and activate ion channels for cell signaling. Metal ions are usually more economical and stable under harsh biomaterial scaffold synthesis and fabrications methods which typically involve the use of organic solvents, free radicals, high temperatures, and pressures which significantly reduce therapeutic efficacy of stimulatory molecules such as recombinant proteins and peptides. Fabrications methodology such as electrospinning and phase separation, and biomaterials such as bioceramics and biodegradable polymers can easily adapt to the incorporation of metallic ions as a therapeutic agent. These biomaterials can readily facilitate the controlled release of metal ions by biomaterial degradation. Researchers have demonstrated novel biomaterial scaffolds composed of biodegradable metals such as magnesium alloys and ions which undergo controlled dissolution in vivo, thereby yielding biodegradable metals implants and coatings

for applications such as sensors. Copper, strontium and zinc are cofactors in metabolic processes of bone and immune system, and in particular are being explored for bone tissue engineering due to their role in bone pathological conditions. Metallic ions can overcome several challenges such as drug decomposition, in stability, bacterial adhesion to biomaterials, and improving host-implant integration at reduced cost, safety and significantly reduced risks. The success of new treatment strategies using a combination of novel conductive biomaterials and metal ions will involve significant collaborations between biologists, materials scientists, biomedical researchers and tissue engineers.

6 Conclusion

The bionic hand is still very much experimental and several challenges need to be overcome such as new lightweight materials, stimuli responsive materials, electrically conductive biomaterials for implantation and integration, tissue regeneration, and new technological advances promoting reliable recording and stimulation of nerves. Following prosthetic fitting, the subjects must undergo training for controlling limb prosthetic movement with periodic adjustments and adaptation based on sensory and tactile feedback. The field of neuroprosthetics holds significant promise in revolutionizing the options for amputees especially in providing a functional replacement and regenerative device that interfaces the biological process with robotic components composed of shoulders, elbow, wrist and the digits. The regenerative toolbox will facilitate convergence of discrete disciplines to effect guided tissue regeneration using components such as scaffolds, controlled surface topographies, stimulatory cues, both chemical and physical factors, and their integration with robotic systems, to restore limb functionality. Continued funding from several sources including government organizations and private foundations will foster synergistic collaborations between, engineers, chemists, biologists, material scientists, clinicians and physiotherapists to make bionic limbs a common and affordable reality.

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Fig. 1.
Body-powered prosthesis **a** and myoelectric prosthesis for shoulder disarticulation amputees **b**.

Fig. 2. Conceptual schematic of targeted motor re-innervation **a** and median nerve dissected during targeted muscle reinnervation (TMR) surgery **b**. Note: short distance required for coaptation of median nerve to motor point of medial head of the biceps (*), and length of nerve end that will be discarded after coaptation (**)

Fig. 3.

Direct nerve interface electrodes for recording and stimulating nerve fascicles. Extraneural electrodes surround nerve and record/stimulated from surface; variations include **a** nerve cuff electrodes, **b** flat-interface nerve electrodes, and **c** photograph of nerve cuffs implanted on rat sciatic nerves for acute experimentation. Cross section of these cuffs providing for easy implantation and approximately 270° of circumferential contact around nerve.

Fig. 4.

Example images of intra-neural electrodes: **a** Cyberkinetics silicon-based 100-channel multi-electrode array (MEA), **b** view of recordings sites on Cyberkinetics array, **c** neuronexus silicon-based MEA shanks, **d** Tucker-Davis Technologies (TDT) microwire MEA, **e** view of recording sites on TDT microwire array, **f** Moxon thin-film ceramic-based MEA, and **g** view of bond pads on a 36-channel Cyberkinetics array.

Fig. 5. Electrode array on a silk film on a feline brain. Controlling humidity of film allowing it to become conformal and consents to wrap and transfer electrodes on curvilinear surfaces such as tip of a test tube.

Fig. 6. Skeletal muscle microtissue attached to elastic cantilevers and selectively stimulated with blue light **a** and depending on area of stimulated tissue region, either whole tissue construct contracted, or only one of its parts **b**. Distance between cantilevers being $\sim 300\ \mu\text{m}$.