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The role of osseointegration in bionic prostheses of the upper limb

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SUMMARY

Background

Amputation treatments are often demolitive, performed in urgency or even in emergency. For this reason, the stump creates by the amputation could result poor functional and painful, and this could considerably hinder the success of the prosthetic treatment (*1*).

Amputation can occur secondary to multiple conditions, including trauma, tumors, infection, and peripheral vascular disease. The main events responsible for limb amputations in the developed countries are disvascular disease, trauma, and war-related damages. Currently, the amputation field in the USA has gained higher attention because it represents up to 7.4% of primary injures involving young and highly functioning people, especially because of war. In last years, there was an intense development of innovative prostheses and new control strategies, therefore it is crucial the issue of functional preparation of the stump for the use of the robotic advanced prosthesis aiming to increase the success of the prosthetic-rehabilitative treatment.

The functional recovery of the amputated limb depends largely on the surgical management of the amputation. It is not infrequent that many amputations require a revision of the stump. Traditional techniques are characterized by principles that are not changed in the last two centuries: preserving the maximum length of the limb, neurectomy, and soft tissue configuration to create a padding for the prosthetic socket (*2*).

The philosophy behind the traditional technique is to create an ideal accommodation for the stump on the socket, conceiving the stump as a passive support for the prosthesis. This led to great difficult in the aim to create a stump capable to control an advanced robotic prosthesis. For example the classic technique of neurectomy and burying in the muscles can lead to the formation of painful neuromas, as a result of an uncontrolled attempt by the nerve fibers to regenerate and makes the peripheral nerves inaccessible for a future surgery (*3*).

Given the progresses of the new generation prostheses that provide an increased number of functions that the amputee must be able to control, the revision of the stump acquires a new and further importance. Indeed, the recent developments of new control strategies and prosthetic components aimed not only at the functional replacement of the lost upper limb, but also at its integration into the patient's body scheme (embodiment) (*4*).

The new advanced prostheses have many degrees of freedom to control, it is therefore mandatory that the patient has the possibility of using an effective control to a large amount of information.

Motorized hand, wrist and elbow prostheses are commercially available, controlled by surface electromyogram (EMG) extracted from a residual pair of agonist-antagonist muscles. This method commonly allows an isolated movement of one joint, but not the coordinated movement of multiple joints, such as a contemporary elbow flexion and hand grip. Achieving a high-quality control of this

type of prosthesis is very difficult, especially for those subjects with bilateral and/or above elbow amputations, where the disability is greatest. These people need to have prostheses that replace the hand, wrist, elbow, and sometimes even shoulder, but do not have enough residual muscle to extract the necessary EMG signal from.

Indeed, the subject is required to apply a stratagem, using residual muscles that were originally used to control different movements and translating their contraction into grip and elbow control. Therefore, non-homologous ways are exploited and the subject is forced towards a "non-natural" motor re-learning (*5*).

A way to solve problems related to prosthesis control in patients with proximal upper limb amputations has developed thanks to the Targeted Muscle Reinnervation (TMR) technique, developed by Dr. Todd Kuiken and by Dr. Gregory Dumanian.

The surgical technique of TMR consists in the transfer of the remnants of the main nerves of the upper limb towards new muscle targets that have lost their original function; for example the pectoralis major in patients with shoulder disarticulation. Before being reinnervated, the target muscles are denervated by their native motor innervation, so the transferred nerve can reinnervate them more easily. The target muscles are then used as "biological amplifiers" of the nerve signals of the motor nerves that originally controlled the amputated district. By transferring more motor nerves of upper limb to different areas of the pectoralis major, the myoelectric signal taken after TMR allows a simultaneous and more intuitive control of advanced prostheses with a high number of active joints (*6*).

Furthermore, initially in a casual fashion and then in a more structured way, it was also seen how the sensory component of the transferred nerves recolonized muscles and overlying skin, causing a tactile stimulation on the reinnervated muscle to be perceived by the patient as coming from the amputated segments. The sensory component of TMR, has been defined as Targeted Sensory Reinnervation (TSR).

The anatomical areas corresponding to the muscles reinnervated during TMR, if appropriately stimulated, can also be used to transmit tactile information relating to the missing limb, since the relocated nerves are composed of both efferent and afferent fibers. In fact, it has been shown that, through TSR (Targeted Sensory Reinnervation), it is possible to restore a tactile sensation relating to the missing limb by stimulating the re-innervated anatomical area (*7*). This could extend the use of the method towards the control of "closed loop" prostheses, where motor control and sensory feedback unite the prosthesis and the patient through an action-perception-action link, typical of the normal motor control of physiological systems (*8*).

Another important factor in determining the success of a prosthesis is related to the comfort during its use. Following amputation, the interrupted nerve terminals deprived of their physiological target can give rise to abnormal benign growths of the nervous tissue (neuromas) which, irritated by mechanical or chemical stimulation, usually cause severe pain to patients. Neuroma pain not only affects the quality of the prosthetic experience (the pressure reflected on the neuroma causes non-physiological discharges), but also, more generally, the amputee's quality of life. Between 25% of individuals with major amputations and 70% of those with traumatic amputations develop chronic localized pain following symptomatic neuromas. Neuromas are very difficult to treat pharmacologically and surgically undergo a high rate of relapse.

TMR can prevent or reduce neuroma formation by providing the dissected nerves with denervated muscle as a target for their regrowth, thus enabling guided axonal regeneration (*3*).

Furthermore, the common employment of prosthetic custom-design socket is characterized by discomfort due to sweat in the prosthetic device, sores or skin irritation, incapacity to walk without any types of limitation in unstructured environment and open fields (*9*). Osseointegrated prosthetic devices for limb amputation are changing to overcome the barriers of custom-design socket. The osseointegration surgical technique for the attachment of prosthetic limb has been used since the 1990s, but nowadays its applied in various fields such as total joint replacements, dental implants, the edentulous mandible, craniofacial deficiencies, maxillofacial reconstruction, orbital prostheses, and bone-anchored hearing aids. Osseointegration is defined as the direct anchorage of an external prothesis to the skeleton in selected patients with amputations. It represents a valid possibility able to provide direct structural and functional link among the bone and the artificial devices, offering the potential for enhanced biomechanical advantage and rehabilitative potential (*10*). In addition to eliminating socket-associated complications osseointegration carries many potential benefits for patients who tolerate their socket systems poorly, including reduced energy expenditure, improved range of motion, walking ability, and sitting comfort, as well as indirect sensory feedback through a complex mechanism termed osseoperception. The osseoperception is defined as the improved ability to distinguish vibratory sensations of the prostheses perceived via the implant directly through the bone. Probably the loads connected directly to bone thanks to the osseointegration process improve the sensory feedback that should help the control of artificial limb (osseoperceptive sensory feedback). Despite the touch has been considered the main sense for osseoperception mediation, several studies have shown as osseoperception is a multisensory perception, based on activation of the central nervous system. This point is essential to justify amputees' improved environment perception with the OI devices and their embodiment process. Embodiment is the perception that something not originally belonging to the self becomes part of the body. Already today, it is possible

to state that osseointegration and osseoperception improve prosthetic control, prostheses perception and adaptation supporting the creation of a new reinforced image of themselves in which the external device became part of their body. Bodily incorporation of the prosthesis has significant repercussions on the social and psychosocial adjustments of the patients themselves, giving functional improvements and reserving existential implications in the concept of quality of life (*11*).

Part 1 - Orthopaedic Osseointegration: State of art

Background

Extremity amputation can be due to several different causes. In developed countries, the leading conditions responsible for amputation are certainly malignancy, infection, and peripheral vascular disease, although trauma and war-related injury remain the main etiologies in the developing world (*12*) (*13*) affecting young, highly functioning individuals and leading to high levels of disability (*14*). Amputation defines a permanent modification to limbs anatomy and function, causing a permanent risk of physical and psychological impairments, as well as social, vocational, and recreational activity reintegration barriers. Despite the considerable technological improvements of the modern prostheses, a patient with an amputee limb remains limited and often incapable to return to his previous level of activity. Traditional prostheses are characterized by a socket fitted over the residual limb through compression or suction-based and suspension systems. These kinds of implants are related to the development of several complications: suboptimal fit, dermal problems, such as skin breakdown, persistence of pain in the residuum (*15*) (*16*). Osseointegration is an innovative surgical approach that permits the anchorage of an external stump directly to the skeleton. This is possible thanks to the apposition of bony tissue exclusively around the device, without the growth of fibrous tissue at the bone-implant interface. Osseointegration is reserved for amputees that do not tolerate conventional sockets. In these patients, osseointegration represents an alternative solution characterized by higher biomechanical advantage and greater rehabilitative potential.

From the origin to the limb amputees.

The Swedish researcher Per-Ingvar Brånemark was the pioneer of the osseointegration mechanism. Around the 90s, he accidentally discovered that the titanium chambers implanted inside a rabbit tibia were remained perfectly soldered to the bone at the end of the experiment, in the absence of soft tissue reaction or loosening (*17*). This observation laid the groundwork for the employment of

titanium implants as an innovative solution to restore bone loss. Starting from his rabbit experiment, Brånemark made several successful investigations on the intraosseous anchorage of dental prostheses (*18*), conducting in 1965 his first human trial in an edentulous patient (*19*). The great long-term results of his clinical trials proved the functional and structural advantage derived by the connection between the living bone and the titanium implant. Brånemark himself later defined this phenomenon "osseointegration".

The introduction of osseointegration mechanism for amputees began in the 90s, thanks to biomechanical studies conducted by Brånemark's son. Richard Brånemark analyzed the efficacy of bone-anchored implants in in healing, post-irradiation, and in experimental arthritis in rat, rabbit, dog, and human (*20*) giving the basis for implant strategies and rehabilitation protocols for limbs osseointegrations.

The first bone-anchored implant in a human amputee was realized in May 1990 on a 25- year-old woman, undergone bilateral transfemoral amputation at the age of 15 as consequence of a tram accident. She was undergone to a two-stage procedure: the first one for the implantation of a titanium fixture directly connected to the skeleton; the second one, six months after, for the introduction of a titanium abutment where attached the stump. The same procedure was obtained for the right and the left leg over a year. An intensive period of rehabilitation succeeded the surgery, at the end of which the patient resulted able to walk with crutches and exercise with cycling. Other clinical experiments were made on transfemoral, thumb and transradial amputees for all the 90s.

Strengthened by the experience of his results, R. Brånemark decided to realize a standardized protocol for the implant system, surgical technique, and postoperative rehabilitation successively named OPRA (Osseointegrated Prostheses for the Rehabilitation of Amputees). OPRA protocol was firstly employed for femoral implants (1998), followed by forearm (2003), humeral (2003), and thumb systems (2005). Still today, the OPRA system represents the leading protocol for standard operating procedures and rehabilitation for femur, humerus, forearm, and thumb amputees (*21*).

Biology of osseointegration

The success of osseointegration depends on the biological ability of the bone to incorporate a nonbiological implant creating a direct structural and functional connection enable to support physiologic load bearing.

The osseointegration process starts with the activation of an osteogenic mechanism mediated by several intracellular and extracellular biologic events. The fixture placement determines the hematoma development at the bone interface essential for clot formation at the surface of the implant (*22*). Platelets that compose the clot undergo several changes stimulating the production of a fibrin

matrix that acts as a scaffold for osteoprogenitor cells to differentiate into osteogenic cells. These cells create a calcified matrix on the surface of the implant that promotes woven bone formation subsequently transformed into lamellar bone (*23*). This construct defines the early biologic fixation. The bone directly linked to the implant responds to mechanical stimuli and undergoes morphologic re-modelling like the host bone. Osseointegration can be promoted or limited by many factors (*24*). Implant characteristics, quality of the host bone, use of pharmacological agents have all been involved in the osseointegration process. Undoubtedly, titanium represents the best material to employ for a successful osseointegration thanks to its biocompatibility, its ability to resist corrosion, and its power to create a surface oxide layer that supports mechanical loads (*25*). Roughened surfaces should be preferred because, increasing platelets adhesion, make the process of bone integration easier (*26*). The stability of the implant is also extremely significant. Excessive micro-and macro-movements at the bone-implant interface inhibit the peri-implant bone formation and favor the development of a fibrous membrane that can lead to aseptic loosening and failure of the implant. As with any external device, osseointegrated implants are exposed to infections. For this reason, their employment in patients affected by immunosuppressed conditions is not recommended. A compromised immune system could reduce the longevity of the procedures, bringing to definitive failure of the implants (*27*).

Patients' selection

The great success of osseointegration largely depends on the choice of patients. Patients must have adequate characteristics, ranging from their health status to compliance, for an optimal result of the implant functioning (*28*). For this reason, although without a worldwide consensus inclusion and exclusion criteria have been defined. Patients candidate to OI implants must have been skeletally mature, have adequate bone stock in the residual limb, be compliant in the performance of the rehabilitation protocol, be in good overall health (*29, 30*). Despite all the percutaneous osseointegrated systems should be reserved for patients which experienced socket complications, today the Food and Drug Administration (FDA) admits the use of OI devices also for patients with expected complications (*31*). Patients affected by skeletal immaturity, infection, vascular disease, diabetes mellitus, osteoporosis, metabolic bone disease, untreated skin disease, as well as smokers or patients who undergo chemotherapy or immunosuppressant drug treatments should be excluded by the protocol of OI. These conditions compromise the survival of the implants predisposing to the risk of superficial and deep infection and/or limiting the bone ingrowth.

Surgical Technique

A standardized surgical technique for osseointegration implants in amputees still does not exist. However, all approaches present several similarities. Preoperative planning is essential. The surgeon should always evaluate soft tissues integrity and the quality of the residuum both clinically both radiologically (*32*). Plain radiographs are necessary to define the bone length and quality. CT scan could be employed to exclude severe osteoporosis and to determine the endosteal diameter and cortical thickness.

Osseointegration technical surgery can be performed in a single (*33*) or into two stages (*34*). In the last case, a period ranging from 6 weeks to 6 months (*21*) between first and second procedure should be considered to permit skin and soft-tissue complete recovery and stable and safe osseointegration. The first surgical step foresees the preparation of the medullary canal for implant placement. The intramedullary component is put into the distal aspect of the residuum bone. The main factor that prevents micromotion and, thus, implants failure is the prompt stability of the fixture to the endosteal surface when a conventional osseointegration device is employed, or the distal osteotomy surface in case of compressive osseointegration. If a single-stage approach is performed, the skin must be immediately prepared because the device is carried through the skin and later loaded following a specific rehabilitation program. In the case of a two-stage procedure, soft tissue complete healing must be obtained before starting the second surgery. During the waiting period, socket use without loads is permitted (*21*). The second surgery consists of the direct connection of the fixture to a percutaneous abutment. A prudent control of the surrounding tissues (muscles, skin etc) is required, to create a stable soft tissue construct that reduces the risk of infection. The use of the socket with progressive weight-bearing is allowed after wound healing.

Current Implant Systems

Over the years, several osseointegrated devices have been realized for patients with limbs amputation. Despite all implants having the purpose to guarantee a safe and valid osseointegration mechanism, each of them is characterized by a different specific design. Today, only five OI systems are really used: the OPRA, the compress transcutaneous implant (CTI), the integral leg prosthesis (ILP), the osseointegrated prosthetic limb (OPL), and the percutaneous osseointegrated prosthesis (POP). Although all devices have the same purpose of achieving solid osseointegration and external prosthetic accommodation, design variability is considerable.

Osseoanchored Prostheses for the Rehabilitation of Amputees (OPRA)

The OPRA system (Figure 1) was firstly introduced in 1998 in Sweden with a femoral implant. It is made of a fixture, an externally threaded intramedullary implant responsible for osseointegration, an abutment, a percutaneous implant that allows the attachment of the external prosthesis (Figure 2); and an abutment screw with the function to secure the abutment to the fixture (*35*). The implant is realized in titanium. The OPRA protocol is a two-stage procedure, split by at least 3 months (*36*). Its survival rate remains the most hopeful result (92%), with 45% of revision-free survival and 20% of complications (*37*). The OPRA system represents the primary recognised and available procedure both for upper and lower limbs. It has obtained FDA approval.

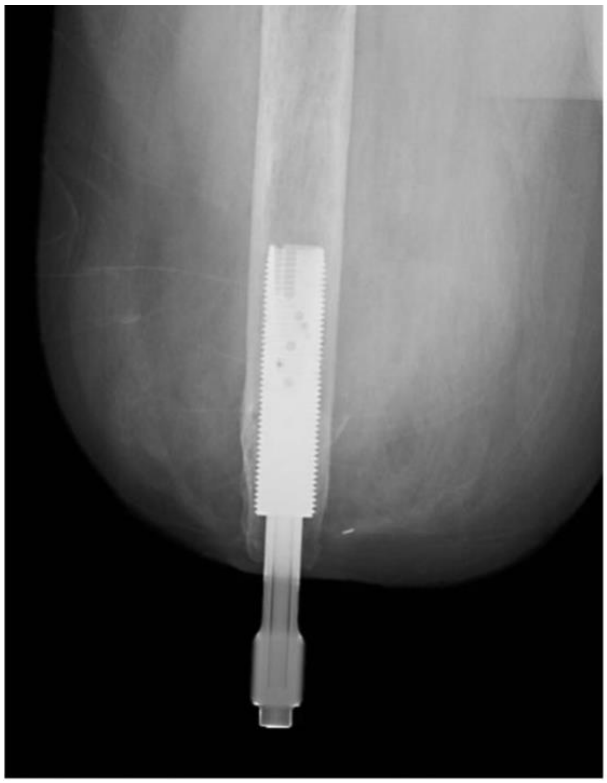

Figure 1: AP femoral radiograph demonstrating percutaneous placement of the OPRA implant

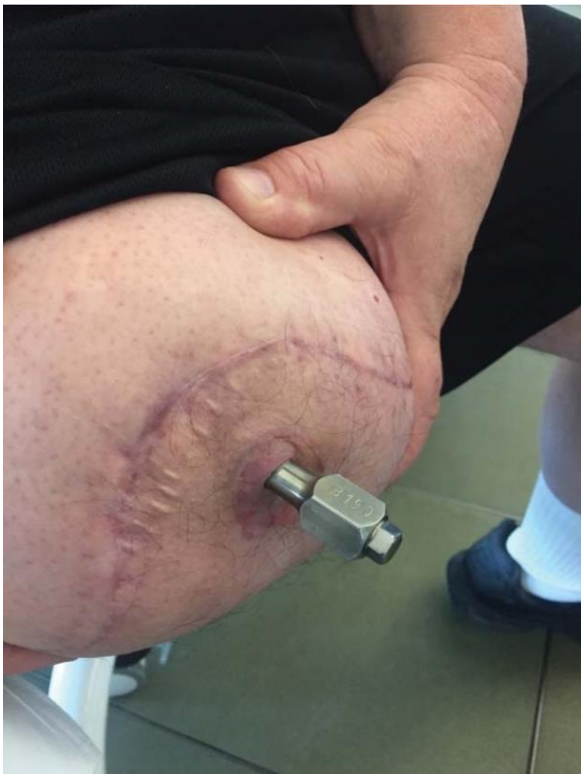

Figure 2: Clinical photograph showing the OPRA percutaneous abutment to which the external prosthesis attaches

Compress Transcutaneous Implant (CTI)

The CTI system (Figure 3), introduced for the first time in 1993, was born as an evolution of the Compress endoprosthesis device originally created for limb salvage procedures (*38*) (figure 2). The CTI consists of an intramedullary anchor plug fixed with transverse pins that are joined to a preloaded compression device at the distal aspect of the residual limb (*39*) (Figure 4). In this way, osseointegration promotion is obtained through the use of elevated compressive loads. As the OPRA, this system can be realized with a single-stage or a two-stage procedure. It is however not yet available in clinics (*40*).

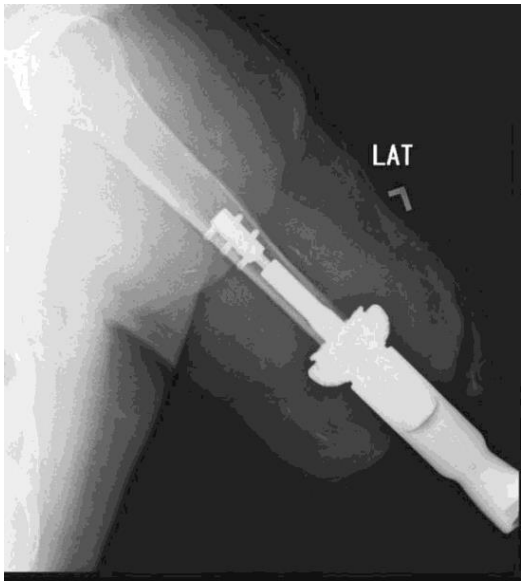

Figure 3: AP humeral radiograph with a CTI system in place. Surgical clips are noted from a targeted muscle reinnervation procedure performed concurrently with the osseointegration procedure

Figure 4: Clinical photograph with the CTI device connected to an external prosthesis

Integral Leg Prosthesis (ILP)

The ILP system, introduced in 1999, was originally created for transfemoral amputees, despite later tibial and humeral applications have been described (*41*). It is made of two major components; an intramedullary endomodule and a transcutaneous bridging connector to the artificial limb, coupled with a dual cone adaptor and a screw. The endomodule possesses a macroporus surface layer to improve osseointegration and depends on a press-fit mechanism of action into the residual bone (*42*).

The ILP has been approved in Germany and the Netherlands, but it has not been acknowledged by FDA.

Osseointegrated Prosthetic Limb (OPL)

The OPL was realized in Australia in 2013. The system has been employed for transfemoral, transtibial, and trans-humeral amputees. It consists of an intramedullary device that is secured to a dual-cone transcutaneous implant with a locking screw. Two different forms of OLP exist, type A and type B. The type A OPL is characterized by an extramedullary implant placed distally, whereas the type B OPL has an intramedullary one (*43*). The OPL has not received FDA approbation for its use in the USA. It is performed only in Australia and the Netherlands.

Percutaneous Osseointegrated Prosthesis (POP)

The POP system was idealized for transfemoral amputees at the University of Utah. The prosthesis has an intramedullary bone-implant region coupled to a subcutaneous collar to which the percutaneous post attaches (*44*). The intramedullary portion of the prosthesis contains a ribbed region proximally and a porous-coated region distally to aid in osseointegration. It is in the progress of evaluation on the FDA Early Feasibility Study started in 2016.

Conclusions

OI-devices represents a hopeful solution to improve amputees' recovery of daily functional activities. Certainly, future research must be conducted towards the optimization of implant properties, from the macro to the nano level, to improve bone ingrowth and reduce aseptic complications of the implants. The most challenge is however focused on the neuromuscular integration of the devices, which could be able to restore volitional motor control, proprioceptive and sensory feedback from the artificial prostheses, particularly in the upper extremity. Concerning the last point, a modified OPRA system, called enhanced OPRA (e-OPRA), has been realized. The e-OPRA permits bidirectional communication between implanted neuromuscular electrodes and the external device thanks to a system known as the Osseointegrated Human-Machine Gateway (*45*). It consists of an OPRA device with modified central and abutment screws, building in a way that nerve electrodes pass transcortically and transdermally. These electrodes can directly communicate with an artificial limb controller, based on advanced algorithms and neural stimulation paradigms to permit volitional motor control and sensory feedback (*46*). Translational research is being directed at the development of biologic controllers that can better modulate bionic signaling in such brain-computer interface devices. Agonist-antagonist myoneural interfaces (AMIs) is another advanced strategy that allows

voluntary prosthetic control and proprioceptive feedback. It consists of the link of muscles with naturally oppositional functions to reproduce native limb dynamics. Other innovative biologic control procedures are the regenerative peripheral nerve interface and the dermal sensory interface 47 48. They are based on the use of a divided peripheral nerve which reinnervates a free muscle or skin graft, respectively. These constructs permit electrodes to transmit functionally selective, high amplitude motor control signals from the brain, and sensory signals to the central nervous system. Although still in its early stages of growth, neuromuscular integration associated with osseointegration will realize more natural prosthetic function and improved results.

Osseintegration: clinical outcomes and complications

Introduction

A limb amputation is a catastrophic event that affects the mobility, quality of life and daily activities of patients. The more common causes for a lower-limb amputation in developed countries are chronic diseases, such as atherosclerosis and diabetes, often associated, whereas in developing countries, traumatic aetiology related to industrial, traffic, and wartime injury predominates (30). The amputation rate associated to military conflicts in the United States Army ranges from 7.4% to 19% of all major extremity injuries sustained, that should potentially shift the prevalence of amputations to younger individuals (*14*, *47*) . On the other hand, upper limb amputation occurs following high-energy trauma, involves young males in good health (*48*), affecting severely the occupational ability and return to work (*49*).

The conventional use of prosthetic custom-design socket is affected by discomfort related to sweat in the prosthetic socket, sores or skin irritation from the socket, inability to walk in woods and open fields, inability to walk quickly (*50*). Therefore, osseointegrated prosthetic implants for limb amputation are progressively evolving to overcome limitations of custom-design socket. The osseointegration surgical technique for the attachment of prosthetic limb is used since the 1990s, but nowadays its application fields are various: total joint replacements, dental implants, the edentulous mandible, craniofacial deficiencies, maxillofacial reconstruction, orbital prostheses and bone-anchored hearing aids (*10*). Osseointegration is a promising alternative that provides direct structural and functional connection between the bone and the artificial metal implant (*51*).

Furthermore, osseointegrated implant may be a reliable option to achieve a bidirectional human-machine interface, especially in upper limb. For instance, "e-OPRA" (Integrum) system allows perimysial and perineural electrodes to pass within the implant, obtaining an advanced motor control of the prostheses and achieving a sensory feedback stimulating the peripheral nerve (*45*). The latter is paramount to obtain a better control and coordination of the robotic prostheses (*52*, *53*) . However,

the osseointegrated transhumeral implant demonstrated its efficacy also in interfaces which do not require electrodes, such as Targeted Muscle Reinnervation (TMR) (*54*) .

Moreover, Lundberg et al (*11*) have demonstrated that the bone-anchored prosthesis contributes to the phenomenon called prosthetic "embodiment" namely that the patients have a higher acceptance of his/her artificial limb that may lead to a sense of independence. Several centres in the world are now performing osseointegrated prosthetic implants. Over the years, changes in implant design, surgical technique, perioperative and postoperative care, and rehabilitation protocols have resulted in improvements in functional outcomes and health-related quality of life. However, the rate of stoma-associated infections remains a concern (*37*). Less common risks are deeper soft-tissue infection, fractures from falls, and loosening of the implant.

The aim of this article is to present a systematic review of these, safety -in terms of rate of infection and complications -and reported outcomes of upper and lower-limb osseointegrated prosthetic implants.

Material and Methods

A quantitative synthesis of all clinical studies of osseointegration technique in case of upper and lower limb amputees was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines with a PRISMA checklist and algorithm (*55*) . The article's search was performed using Medline, Embase, PubMed, Cochrane, CINAHL, and Ovid databases. The combinations of keywords we used was: "Osseointegration", "Upper", "Lower", "Limb", "amputees" and "clinical outcome". Articles published from inception of database to 2020 were selected. Two independent reviewers separately conducted the search. All journals were considered, and all relevant studies were analysed. To be included in the study, articles had to be published in a peer-reviewed journal. Articles without an abstract, were excluded from the study. Screening of the articles was done considering the relevance of titles and abstracts and looking for the full-text article when the abstract provided insufficient information about inclusion and exclusion criteria. The two investigators separately reviewed the abstracts and then performed a close reading of all papers and extracted data, to minimize selection bias and errors. To obtain other articles, the investigators performed a cross-reference search of the selected articles. The last search was performed on 06 February 2020.

According to the Oxford Centre of EBM, Level I-IV articles were found in the literature and included in our study. Articles in Italian, English, Dutch, Spanish and French were included. The criteria used to select articles were:

1) article about upper or lower limb amputations treated with an osseointegrated prosthesis.

2) articles with a detailed description of the surgical procedure, clinical outcome, complications with adequate follow-up period.

Literature reviews, case reports, studies on animals, biomechanical reports, technical notes, letters to editors, cadaver, or in vitro investigations and instructional were excluded from the review. Moreover, articles with insufficient details of surgical intervention, clinical postoperative outcomes, clinical examination, statistical analysis, age of patients, and follow-up were excluded as well.

To reduce and avoid bias, the authors reviewed and discussed all the selected articles, the references, and the articles excluded from the study. If any disagreement was present among the reviewers about the inclusion and exclusion criteria, the senior investigator made the final decision.

Quality Assessment

The quality of included studies was evaluated using the MINORS (Methodological Index for Nonrandomized Studies) score. The following domains were assessed: a clearly stated purpose, inclusion of consecutive subjects, prospective data collection, endpoints appropriate to the purpose of the study, unbiased assessment of the study endpoints, follow-up period appropriate for the study, loss to follow-up of less than 5%, prospective calculation of the study size (**Error! Reference source not found.**). The items analysed were 8, scored 0 (not reported), 1 (reported but inadequate) or 2 (reported and adequate) therefore, 16 is the maximum value for non-comparative studies.

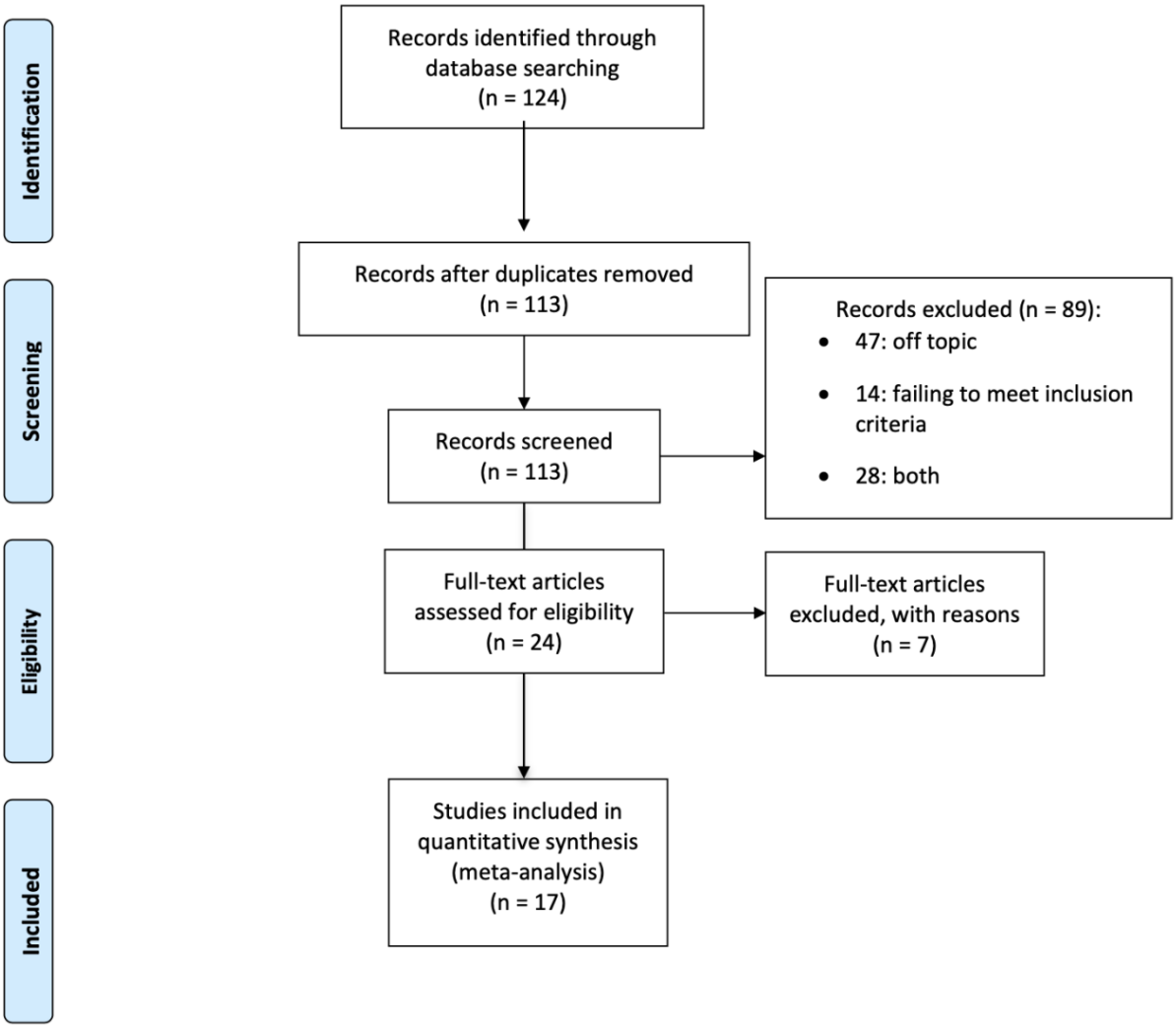

Figure 5: PRISMA 2009 Flow diagram

Results

The literature search and cross-referencing resulted in a total of 124 articles (Figure5). 11 duplicates had been identified and removed, leaving 113 articles. Out of these, 89 were rejected because of off-topic abstracts (47 articles), failure to fulfill the inclusion criteria (14 articles), or both (28 articles). After reading the remaining 24 full-text articles, another 7 articles were excluded because of insufficient details and uncertain diagnosis and outcome measures. Finally, 17 articles focused on the treatment of patients with upper or lower limb amputation treated with an osseointegrated prostesis were included. **Error! Reference source not found.** shows, the search algorithm according to the PRISMA guidelines.

*Demographics (**Error! Reference source not found.**)*

A total of 634 patients with 669 implants were included, with a median age at surgery of 44,7 years ranging from 17 (*43*) to 84 (*56*) years. Several levels of amputation were analyzed: 586 transfemoral, 37 trans-humeral, 24 trans-radial/ulnar, 12 thumb or partial hand and 6 transtibial. The major cause of amputation was trauma for 419 patients (65%). Other less frequent reasons are tumor (15%), infections (4%), vascular diseases (2%) and other causes such as congenital and 4th degree burn (16 %). Patients were assessed at follow-up for an average 61,4 months, ranging from 10 months to 228 months (*37*).

Table 1: Demographic

Authors	**Study Design (level of evidence)**	**Number of patients (limb)**	**Mean Age (years)**		**Level of amputation**	**Cause of amputation**	**Type of implant**	**Follow-up (months)**
Tillander J et al 2010	Case series (IV)	39 (45)	-	49.3 (74-28)	Transfemoral (33), Transradial (4), Transhumeral (3), Transtibial (1)	Either trauma or neoplasia	Not reported	36
Muderis M. et al 2016	Case series (IV)	86 (91)	32 ± 14	48 ± 14	Transfemoral	Trauma (65), tumor (11), infection (8), congenital (1), other (1)	OPL implant system	34 (24 - 71)
Muderis M. et al 2017	Retrospective study (III)	22 (22)	-	46.2 (67 - 20)	Transfemoral	Trauma (16), Tumor (4), Infection (2)	OPL implant system	14 (10 - 30)
Aschoff H. et al 2010	Retrospective study (III)	35 (37)	33 (14 - 56)	44 (17-69)	Transfemoral	trauma (30), tumor (4), other (3)	Endo-Exo-Femurprosthesis	-

Brånemark R. et al 2014	Prospective study (II)	51 (55)	32 (13 - 64)	44 (20 - 65)	Transfemoral	trauma (33), tumor (12), other (6)	OPRA	24
Juhnke D-L. et al 2015	Retrospective study (III)	69 (73)	-	Group 1: 46 (17 - 69), Group 2: 45 (24 - 76)	Transfemoral	Trauma (51), Tumor (7), Infection (3), 4th Degree Burn (1), Other (7)	Endo-Exo-Femurprosthesis	Group 1: 74 (6-144), Group 2: 32 (1-59)
Atallah R. et al 2017	Case series (IV)	5 (5)	-	67 (56 - 84)	Transtibial	Vascular diseases (4), Trauma (1)	customized 3-dimensional-printed ti-tanium implant (AQ Implants)	12 (-)
Hagberg K. et al 2008	Prospective study (II)	18 (18)	-	45 (22 – 62)	Transfemoral	Trauma (12), Tumor (5), Vascular diseases (1)	OPRA	24 (-)
Hagberg K. et al 2014	Prospective study (II)	39 (39)	31 ± 14.8	44 ± 12.4	Transfemoral	trauma (23), tumor (11), other cause (5)	OPRA	24 (-)
Jonsson S. et al 2011	Retrospective study (III)	48 (48)	-	40,9 (18 - 64)	Transhumeral (16), transradial (20), thumb (10), partial hand (2)	Trauma (32), Congenital (3), Tumor (2)	OPRA	180 (-)

Tsikandylakis G. et al2014	Retrospective study (III)	18 (18)	-	42 (19 – 69)	Transhumeral	Trauma (16), Tumor (2)	OPRA	96 (24 - 228)
Matthews D. J. et al 2019	Retrospective study (III)	18 (18)	-	32 (21 - 49)	Transfemoral	-	OPRA	228 (21 - 190)
McMenemy L. et al 2020	Retrospective study (III)	7 (14)	-	28 (24 - 33)	Transfemoral	Trauma	OPL implant system	46 (36 - 52)
Muderis M. et al 2016	Retrospective study (III)	50 (50)	-	49,4 (24 - 73)	Transfemoral	Trauma (32), Blast injury (3), Infection (5), Tumor (8), Congenital (2)	OPL or ILP implant system	21,5 (-)
Sullivan J. et al 2003	Retrospective study (III)	11 (11)	-	-	Transfemoral	-	OPRA	-
Tillander J et al 2017	Case series (IV)	96 (102)	-	43,5 (19–65)	Transfemoral	Tumor (20), Trauma (71), Ischemia (5), Infection (5), Other (1)	OPRA	125,5 (64 - 192)

Van de Meent H. et al 2013	Prospective study (II)	22 (23)	-	46,5 (23 - 67)	Transfemoral	Trauma (20), Tumor (2)	OPRA	12 (-)

*Infections and Complications (**Error! Reference source not found.**)*

Infections and complications were evaluated in 16 and 13 studies (*37*) (*28, 29, 31, 35, 42, 56-60*) respectively. The overall rate of infections was 32% (201/626 patients). All the articles included had specified the gravity of infection distinguishing superficial, localized at the skin (163/201 patients), by deep infection localized at bone or causing septic disease (38/201 patients).

Other postoperative complications were analyzed in 13 studies with an overall rate of 75% (164/216 implants). The most common complications were stoma hyper granulation and implant related complication, including breakage, with a rate of 35% and 27% respectively. Other less frequent postoperative complications were periprosthetic fracture or fracture above the implant (18%), soft tissue redundancy (8%), aseptic loosening of implant (8%) and persistent pain (4%) (Figure 2).

*Outcome Scores (**Error! Reference source not found.**)*

All the clinical scores reported in the included studies were related to lower limb. No clinical data for upper limb was reported. The most common clinical scores used were the mental component score (MCS) and physical component score (PCS) of 36-Item Short Form Health Survey (SF-36) (*43, 46, 56, 57*) and the Questionnaire for Persons with a Transfemoral Amputation (Q-TFA) 48 53 57 58. The postoperative mean value of MCS and PCS SF-36 and Q-TFA was 55,1, 45,4 and 73,8 respectively.

Other less reported clinical outcomes were six minutes walk test (6MWT) (*46*) and the timed up and go (TUG) test with an average value of 388 meters and 11,5 seconds respectively.

Table 2: Infections, complications, clinical outcomes

Authors	**Infections**	**Complications**	**Clinical Outcomes**				
			SF -36	**Q-TFA**	**6MWT**	**TUG**	**Others**

Tillander J et al 2010	5/45: Femur (4), Humerus (1)	-	-	-	-	-	-
Muderis M. et al 2016	21/91	stoma hypergranulation (17), soft-tissue redundancy (14), proximal femoral fracture (3),inadequate osseointegration (1), implant breakage (2), and breakage of the pin used as afail-safe mechanism (25)	Significant improvement in SF-36 physical component summary (p, 0.001)	Significant improvement in Q-TFAglobal score (p, 0.001)	Significant improvements (p , 0.001)	Significant improvements (p , 0.01)	-
Muderis M. et al 2017	15/22	None	-	-	significant improvements, with a mean increase of 128%	mean reduction of 30%	-
Aschoff H. et al 2010	14/37	Of 37 patients, 17 had no complications or minor complications and 20 had >1 revisions;of these 20 patients, 4 required explantation (1 due to intramedullary infection, 2 due to chronic soft-tissue problems, 1 due to failure 7 yr after surgery), 2 successfully reimplanted; 14 of 37 patients had minor revision due to stoma, 12 of which were exchange of coupler	-	-	-	-	-

Brånemark R. et al 2014	32/55	46/51 patients >1 complications with a total of 101 complications: 49 classified as "serious"complications in 39 patients;implant removed in 4 patients (1 infection, 3 aseptic loosening); 5 patients had episodic pain during rehabilitation, without loosening; 4 patients with 5 fractures, 3 in the ipsilateral hip, 1 below the elbow, and 1 vertebral compression; no peri- implant fractures reported; 9 mechanical abutment complications in 4 patients (6 in same patient), replaced with no long-termeffect	SF-36 showed significant improvement in general quality of life (p , 0 .0001)	Q-TFA scores improved (p , 0.0001):prosthetic use, prosthetic mobility, global situation, and fewer problems	-	-	-
Juhnke D-L. et al 2015	Group 1: 23/30 (early infection: 13, late infection: 10); Group 2: 0/39	Structural Failure of Implant (1), Periprosthetic or Pertrochanteric Fractures (5), Implants Explanted (4), Unplanned Intervention Due to Soft Tissue Problems at Stoma (24), Any Unplanned Intervention (29).	-	-	-	-	-
Atallah R. et al 2017	1/5	-	SF-36 MCS: 57,4 (41,2 - 70,3); SF-36 PCS: 40,8 (38,9 - 44,4)	Global: 63,2 (58 - 83,3)	311 (144 - 433) m.	18,65 (6,28- 26,8)	-

Hagberg K. et al 2008	2/18	loosening of the implant (1),broken prosthetic components (1), phantom limb pain and pain in stump muscles(1),	SF-36 MCS: 50; SF-36 PCS: 44	Global: 72,1 (33 - 100)	-	-	-
Hagberg K. et al 2014	-	loosening of the implant (3)	SF-36 PF: 60 ± 21,4; SF-36 PCS: 40,5 ± 9,8;	Global: 76 ± 17,4	-	-	-
Jonsson S. et al 2011	1/48	Loosening of fixtures (3), fracture (1), non - user (2)	-	-	-	-	-
Tsikandylakis G. et al 2014	16/18	Loosening of fixtures (2), Incomplete distal fracture (8)	-	-	-	-	-
Matthews D. J. et al 2019	17/18	Fractures (1), persistent pain (1)	improved significantly betweenpre-operativestatus and 2 and 5 years post-operative time points	significant improvements in all of the main scores, and in two out of the three sub-scores for prosthetic mobility, between thepre-operativeperiod and 2 and 5 yearspost-implantation	-	-	-
McMenemy L. et al 2020	none	broken dual cone (1), periprosthetic fracture (1)	SF-36 MCS: 58,19; SF-36 PCS: 54,5	-	402 m	10.6 (7.4 – 12.1) s.	-

Al Muderis M. et al 2016	21/50	periprosthetic fractures (4), failure of osseointegration (2)	SF-36 PCS: 47,29 ± 9,33	Global: 83, 52 ± 18,4	419 ± 31,44 m.	8,74 ± 2,81 s.	-
Sullivan J. et al 2003	2/11	Mechanical deformation with periprosthetic fractures (2), mechanical deformation (3)	-	-	-	-	-
Tillander J et al 2017	16/102	-	-	-	-	-	-
Van de Meent H. et al 2013	8/23	-	-	Global: 75 (42 - 100)	423 ± 21 m	8.1 ± 0.7 s.	-

SF-36, 36-Item Short Form Health Survey**; MCS,** mental component score**; PCS,** Physical component score**; Q-TFA,** Questionnaire for Persons with a Transfemoral Amputation**; 6MWT,** six minutes walk test**; TUG,** Timed up and Go**; s,** seconds**.**

Table 3: Quality assesment: MINORS score

Authors	**A clearly stated aim**	**Inclusion of consecutive patients**	**Prospective collection of data**	**Endpoints appropriate tothe aim of the study**	**Unbiased assessment of the study endpoint**	**Follow-up period appropriate to the aim of the study**	**Loss to follow up less than 5%**	**Prospective calculation of the study size**
Tillander J et al 2010	2	2	2	2	1	2	2	0
Muderis M. et al 2016	2	2	2	2	1	2	2	0
Muderis M. et al 2017	2	2	2	2	1	2	2	0
Aschoff H. et al 2010	1	1	0	1	1	1	2	0
Brånemark R. et al 2014	1	2	2	1	1	2	2	0
Juhnke D-L. et al 2015	2	1	1	2	1	2	2	0
Atallah R. et al 2017	1	1	1	1	1	1	2	0
Hagberg K. et al 2008	2	1	2	2	2	2	2	2
Hagberg K. et al 2014	2	2	2	2	2	2	2	2
Jonsson S. et al 2011	1	1	1	1	1	2	2	0
Tsikandylakis G. et al 2014	2	1	1	2	1	2	2	0
Matthews D. J. et al 2018	2	1	1	2	1	2	2	0
McMenemy L. et al 2020	2	0	0	2	1	2	2	0
Al Muderis M. et al 2016	2	2	2	2	1	2	2	0
Sullivan J. et al 2003	0	0	0	1	1	1	2	0
Tillander J et al 2017	2	0	0	2	1	1	1	0
Van de Meent H. et al 2013	2	2	2	2	1	2	2	0

Quality Assessment

The MINORS score for all non-comparative included studies ranged from 5 to 13, with a median of 11 (interquartile range [IQR], 9-11). Details are reported in **Error! Reference source not found.**.

Discussion

The most important findings of this review are that the osseointegration technique is associated to a high rate of infection (32%) and other postoperative complications (75%) underling the fact that this kind of surgery is high demanding and requires an important expertise by the surgeon. The most

common infections reported are superficial and rarely need an implant removal (*15, 46, 57*). Otherwise, major postoperative complications include breakage of implant, periprosthetic fracture, aseptic loosening, requiring one or more additional surgeries up to the removal of the implant (*29*). However, the data available from the current literature show that the rate of complications associated to bone anchored prosthesis are similar to those of traditional socket ones which includes skin issues (in 40% of patients), residual pain (in 30% of patients) (59, 60) restricted limb movement and excessive weight of the socket especially in case of upper limb amputees. All of these mentioned problems are related to a high rate of prostheses abandonment (*1*).

Since the distal side of prosthesis is in direct contact with environment, it is very difficult to distinguish between a simply skin reaction and a bacterial superficial infection. To diagnose a true skin infection sign of inflammation has to be correlated therefore with positive bacterial cultures and good response of inflammation with use of antibiotics (*37*). In this way, skin motion around the abutment seems to be a predisposing factor for superficial infections and/or skin reactions of the skin penetration site (*43*). However, bacterial contamination (commensals, mutualistic, or pathogenic and potential ‘‘pathogens’’) has been found in half of asymptomatic patients with this kind of prosthesis (*61*). Therefore, some skin reactions are probably recorded as superficial infections and vice versa in the included studies. For these reasons, a classification system of the skin reactions would be useful to quantify the risk to develop a superficial infection helping the clinician to distinguish a real skin infection or a simply inflammatory skin reaction.

Periprosthetic fractures (18%) and aseptic loosening (8%) are important postoperative complications. Therefore, it is important to perform a close radiological follow-up to detect prognostic signs (*62*). The studies regarding upper limb amputees showed that the radiological modifications of transhumeral amputees are similar to those in transfemoral. However, the distal bone resorption in the humerus is often lesser extent than in the femur and the proximal buttressing is localised at side above the fixture (*62*). This seems to be associated to different forces that act in the femur (compressive forces and bending moments) and humerus (tensile forces and bending moments). All of these radiological changes with endosteal bone resorption are potential factors associated to periprosthetic fractures or reduction of fixture's stability.

Even if the high rate of complications and infection seems to discourage those procedures, most of the studies included reported significant improvement in clinical outcomes compared to preoperative time (*46, 56*). These findings agree with the current available literature showing an enhanced comfort, increased range of motion, improved mobility compared with socket-suspended prostheses (*13*) . Regarding upper limb osseointegrated prosthesis, none of the included studies report clinical outcomes. Nevertheless, as above mentioned, trans-humeral amputees achieves good results

especially in terms of full freedom of motion of shoulder joint compared to traditional socket prosthesis (*35*). This demonstrates that this procedure helps the patient to become more independent during the daily activities and avoid the discomfort of prosthetic custom-design socket. Moreover, the osseointegrated prostheses have less maintenance costs over a long time period since, being bone-anchored, there is no need to modify periodically the socket in relationship to residual limb volume, which could change over time (*63*).

Whereas these procedures are associated to a high percentage of complications, the right selection of patient is crucial. The indications and contraindications for this surgical technique are not always stated in the current available literature. Generally, patients have to demonstrated discomfort with the conventional socket prosthesis and they must have an adequate bone stock in the residual limb and must be in good overall health and able to comply with the rehabilitation protocol (*61*). The contraindications are represented by the clinical conditions associated to an increased risk to develop a postoperative infection, fracture or loosening, such as active infection, peripheral arterial disease, diabetes mellitus, current chemotherapy or immunosuppressant drug use, smoking, osteoporosis, metabolic bone disease, or untreated skin disease of the residual limb (*31*).

The osseointegrated implant, besides giving a mechanical stability and avoiding the socket issues, are capable to provide a sort of sensory feedback, called "osseoperception". This may be a further component that should explain the good postoperative clinical outcome of these implants. During last decades, few psychophysical tests have been performed on osseointegrated prostheses confirming a better tactile function that leads to a better physiological integration of the limb (*64*). This seems to be realized thanks to a partial sensory substitution of vibrations and torques (*65*). Which kind of receptor groups involved in this so-called "osseoperception" is still debated in the scientific community. Non-invasive analysis such as Somatosensory Evoked Potentials (SEPs) and functional Magnetic Resonance Imaging (fMRI) are currently being used to help visualizing the activity centres involved in the pathway form the stimulation site to the cortex. Both these approaches provide new standpoints for osseoperception research and would try linking the anatomical and histological background to the clinical observations. If such links are present, osseoperception might help in physiologic and functional integration of implants in the body (*64*).

This work presents several limitations: the studies included are all case series and retrospective with except of two studies (*57*), and was no randomized and comparative showing a low level of evidence, as stated with MINORS score. Moreover, the population included varies widely in age range and several types of implants were used with a relatively short follow-up, making difficult to point out which is better. Only one study (52) have reported the timing of development of infection. Furthermore, studies regarding upper limb did not report clinical outcome results.

Conclusion

The bone-anchored prothesis is associated to a relatively high rate of postoperative infections and complications, but good postoperative clinical scores are highlighted. Furthermore, the osseointegration technique is not related to important socket – related problems (skin sores, excessive sweating, fitting issues etc..) that often lead the patients to abandon the use of a prosthesis.

Therefore, it is a promising technique that would help the patients to carry out a life as close as possible before the amputation, including the possibility to have a better embodiment and to apply a bionic prosthesis. At the same time, it is a high demanding surgical technique that requires important expertise by the surgeon, therefore this kind of surgery should be carried out only in specialized centres.

The data available from the literature are poor, therefore we encourage to perform more clinical studies to report and clinical data, to establish which kind of implant is associated to higher clinical performance and lower rate of postoperative complications and infections.

Osseoperception and Embodiment

Introduction

Although bionic limb replacement was devised since the 1940's, artificial limbs are still far from the functionality of their biological counterpart, moreover, technology used since the 1960's is still considered state-of-art (*66*). Myoelectric control strategies established decades ago, such as the activation of an entire group of muscles (e.g. flexors) to control the direction of a single degree of freedom (e.g. hand close) with the use of surface electrodes, are presently the most sophisticated solution available to patients. EMG signals recorded by surface electrodes, currently used to control advanced prosthetic devices are inconsistent and unreliable. Surface electrodes are limited because of poor skin contact, residual limb sweating and their ability to only record signals from superficial muscles, whose function does not relate to the intended prosthetic function.

As today, prosthetic hardware is considerably more advanced than the control options. There are commercially available myoelectric devices to substitute elbows, wrist, and hands with individually actuated fingers; however, since the patients can hardly control one of these units at the time, they are rarely used all together. The current state of prosthetics can be attributed to the following unresolved issues:

1. A lack of mechanically stable attachment of the prosthesis to the body.

2. A lack and instability of physiologically appropriate signals to precisely control several degrees of freedom (motions) on the prosthetic device.

During the recent 20 years, extensive basic and applied research on orthopaedic osseointegration has been pioneered by Associate Professor Rickard Brånemark and co-workers, resulting in the Osseo integrated Prosthesis for the Rehabilitation of Amputees (OPRA) (*67*). This implant system is now regarded as the best documented mechanically stable attachment of limb prosthesis to the human body. Over 400 patients have been treated with the OPRA Implant System in different countries in Europe, Australia and South America. The OPRA Implant System is a CE-marked medical device.

The mechanic attachment of prosthetic devices to the human body has been the focus of much research and development in recent years (*28*). Ideally, it should provide a stable connection, which allows for physiological weight-bearing and unrestricted range of motion in the adjacent joint. Sockets are still the standard of care in upper and lower limb prosthetics, although they exhibit various shortcomings. Contact between skin and socket may lead to skin irritation and ulceration, as well as increased sweating (*68, 69*) . This also substantially interferes with interface reliability.

In case of short stumps, socket fitting often requires additional fixation, limiting the range of motion in the adjacent joint. Attachment issues, as well as problems with interface reliability, contribute to the high rate of prosthesis abandonment (*1*). To overcome these issues, Rickard Brånemark and colleagues have pioneered the approach of osseointegration (*28, 35*). Hereby, a special titanium implant, with a transcutaneous abutment for prosthetic fixation, is surgically inserted into the bone. Osseointegration, initially designed for lower limb amputations, was first performed in a young woman with bi-lateral transfemoral amputation in 1990 (*21*). Since then, it has increasingly become an established procedure, with standardized protocols for surgery and rehabilitation (*34*). It has been employed in transhumeral, transradial, transfemoral, transtibial, and various hand and finger amputations. Various authors report good functional outcome and high patient satisfaction, with only few adverse effects (*32, 46*) Possible complications include implant failure due to loosening of bone fixation and bone fracture during implantation (*37*). Furthermore, the transcutaneous implant is a predisposing factor for stump infection. However, despite frequent colonization by pathogenic bacteria, only few infections leading to major adverse events such as implant removal, have been reported (*30*). Thus, as underlined before, a secure attachment to the remaining limb is achieved, which results in significantly improved stability in comparison to conventional sockets and the socket-mediated skin problems are prevented.

Osseoperception: a multisensory experience

One of the major successes of osseointegrated implants would seem to be a greater ability of the amputee to live with the prosthetic limb, compared to a suspended alveolar prosthesis. The premise of this innovation arises from a new revolutionary concept named osseoperception.

The osseoperception is defined as the improved ability to distinguish vibratory sensations of the prostheses perceived via the implant directly through the bone (*1*).

The phenomenon of osseoperception was first identified with the benefit of improved sensory feedback for bite force and oral function for patients with osseointegrated implants in the edentulous jaw. In the case of bone-anchored implants, there are histological, neurophysiological, and psychophysical evidence that a proper peripheral feedback pathway can likely be restored by loading osseointegrated implants (*64*). Despite the underlying mechanism of the phenomenon of osseoperception being a matter of debate, patients with bone-anchored amputation prostheses can experience sensory perception, reporting that the prosthesis feels more like their own limb. The physiological integration of implants appears to be of great importance for the patients to provide a more natural function. Osseoperception is vital for both improved prosthetic function as well as for reducing the feeling of being disabled for individuals treated with OI prostheses.

Probably, the loads connected directly to bone thanks to the osseointegration process improve the sensory feedback that should help the control of artificial limb (osseoperceptive sensory feedback) (*21*). The term osseoperception has been stated to imply "the mechano-sensibility associated with osseointegrated implant rehabilitation," further defined as "the sensation arising from mechanical stimulation of a bone-anchored prosthesis, transduced by mechanoreceptors that may include those located in muscle, joint, mucosal, cutaneous and periosteal tissues, together with a change in central neural processing in maintaining sensorimotor function" (*70*). Clinical observations have shown that patients can experience and identify various types of mechanical loading through OI implants. This suggests that a neurophysiological peripheral feedback pathway can be partially restored (*64*, *71*). This hypothesis is supported by results showing that two types of stimulation (pressure and strokes of a toothbrush) of an OI thumb prosthesis resulted in cortical activation on both sides of the somatosensory cortex, evidenced by functional magnetic resonance imaging of the brain, showing that osseoperception is based on activation of the central nervous system (*72*). Moreover, Clemente and colleagues (18) show that not only touch but also hearing is involved in this phenomenon demonstrating therefore that osseoperception is a multisensory perception, which can explain the improved environment perception and embodiment process of bone-anchored prosthesis users (*73*).

Embodiment: Protheses as part of me.

Already today it is possible to state that osseointegration and osseoperception improve the quality of life of amputee patients, prosthetic control, and body image, contributing to the development of a much more complex process called embodiment. Embodiment is the perception that something not originally belonging to the self becomes part of the body (*74*). The feeling embodiment for a prosthesis may counteract amputees' altered image of the body and increase prosthesis acceptability (*75*). The embodiment process is based on a complex interaction between three aspects: return to normalcy, sensation experience and self-efficacy (*73*) (Figure 6). Prosthesis embodiment, or the experience that the prosthesis is a part of oneself, is created directly through the perceived naturalness of the sensory experience, through the modality of the sensory percepts, and a greater sense of confidence in abilities. Confidence is enabled using sensory information in everyday life. The naturalness of the experience of having a prosthesis that can give sensorial feedback transforms the prosthesis from an extracorporeal tool into a normal, animate limb. Having sensory information that was useful in performing tasks yields increased confidence in their abilities to do tasks with the prosthesis. Higher confidence supports the perspective that the prosthesis is a capable and rightful part of the body. The reduced focus and visual attention required to operate the prosthesis, facilitated by the usefulness of the sensory information, further strengthens the perception that the prosthesis is a natural part of the body. The concepts exposed so far are essential if we consider the role of embodiment in terms of social interactions.

Body image represents an essential concept for the social and psychosocial adjustment in amputees. As widely shown in literature, amputees' desire to have a prosthesis perceived like a "body structure" is higher than the permanent sensation to wear an "extracorporeal structure" or an "inert supplement" to the body sensation (*76*). Patients prefer the use of an implant perceived as part of one's body, thanks to bodily incorporation.

Bodily incorporation of the prosthesis presumes, as the main condition, the perception of the prosthesis as a "knowing body part" (*77*). As strongly supported by several current studies, the "knowledge" of the prosthesis is provided by the sensory stimulation, which improves the mechanism of incorporation. One of these studies has shown importance of tactile and proprioceptive signals are critical in establishing body property through the rubber hand illusion experiment (*78-80*) . The strict relationship between sensation and embodiment has also been demonstrated through many kinds of research conducted on amputees treated with targeted sensory reinnervation, sensory feedback provided via electrical stimulation, proprioception provided through the surgical coupling of agonist and antagonist muscles, on upper limb prosthetic users who had experienced vibrotactile or

mechanotactile sensory replacement on the residual limb. Each of these patients underlined that sensation was very important in establishing prosthesis incorporation (*81*) (*82-84*)

The significance of the phenomenon of the embodiment has been largely analyzed by a qualitative and phenomenological study focused on OI prostheses users and their personal degree of incorporation in their prostheses. Each enrolled patient has been questioned through an audio-taped in-depth interview about his personal experience with OI-device compared to previous his standard suspended prostheses. The encouraging results support the role of sensation in prosthetic incorporation for amputees. All participants described their personal experience with the OI prosthesis as a revolutionary change in their lives, documenting improved proprioception, the ability to feel through the skin of the residual limb that was no longer wrapped in a grip, and experiencing the sensation transmitted by the bone through osseoperception. Among the surprising results, they described the ability to trust the fit and suspension of the prosthesis, the capacity to sit comfortably and not requiring consuming time and mental energy living with a socket. All these aspects had contributed to a higher improvement in quality of life, making participants were less frustrated and more active in their social life (*11*).

However, the most important result derived by this research consists in the observation of a self-development process in amputees.

Living with the OI prosthesis had a profound influence on their lives, opening several new possibilities that they had never experienced with the previous stumps.

With the expression "self-development process", patients referred to a gradual revolution in patients' identity: from considering themselves as being disabled to having an identity, to be a potential person. Patients' subjective perceptions with their OI-prostheses were categorized into three different typologies, 'Practical prosthesis', 'Pretend limb 'and 'A part of me'. Among these, the last represents the main revolutionary typology, which mainly summarized the embodiment process. Inside this typology, the amputees described their OI prosthesis as an integral part of the body. They had complete trust in their new device, in its functionality and technical characteristics. The OI-prosthesis reinforced amputees' sensation of being a 'whole body', influencing their vision of the outside world and making them active protagonists of their lives. Presumably, the substantial anchorage of the prosthesis to the skeleton as well as the direct contact between the device and the skin of the residual limb, have largely contributed to the feeling of artificial limb bodily wholeness.

Patients themselves have described as the actual bone-anchorage has reinforced the feeling of being an entire human being, like the person that they were before the amputation. They completely accepted the new device, sometimes forgetting about it (*11*).

'I don't think about having the prosthesis in that it doesn't feel like a prosthesis. With this kind of technology, you can't feel it."

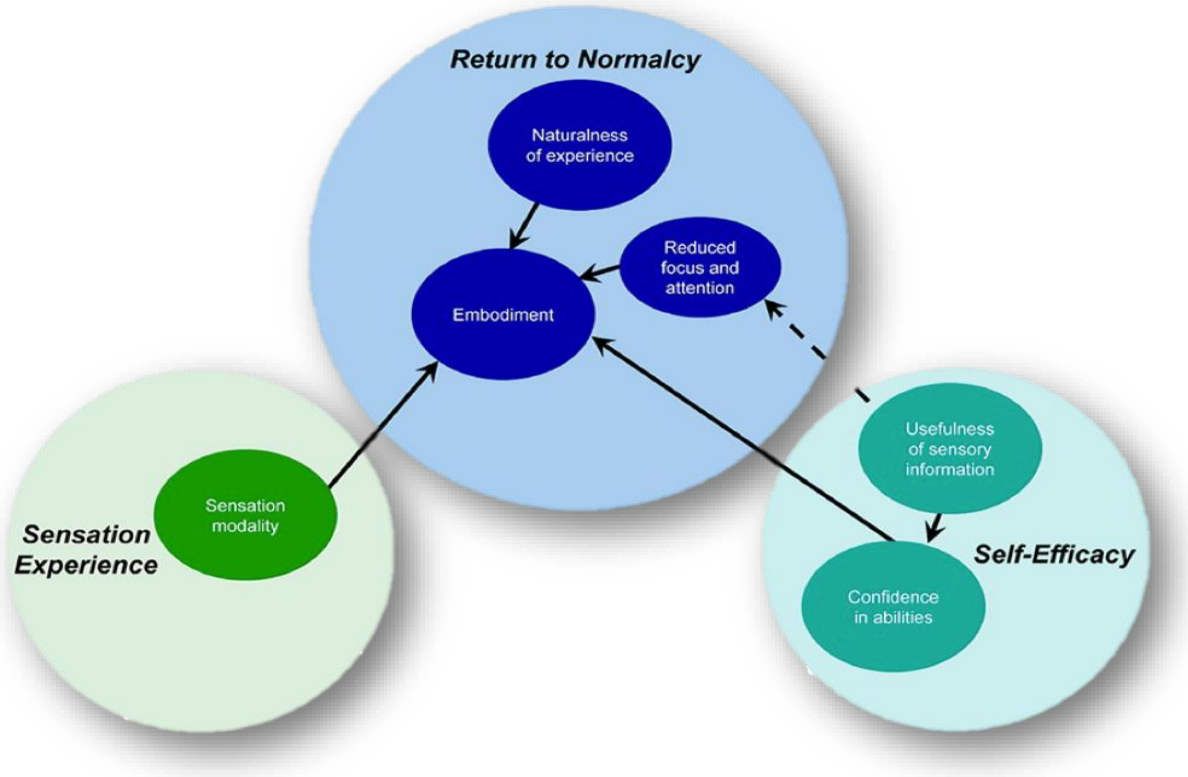

Figure 6: Embodiment Process

Conclusion

Osseointegration undoubtedly contributes to prostheses success, strengthened their acceptance thank to the phenomenon of osseoperception and its essential contribution in the embodiment process. It's essential to underline that the perception of prostheses as an essential and integral part of the body brings radical changes beyond the functional improvements, including existential implications in the concept of quality of life. The future challenge, and our study's aim, is to better understand the mechanisms based on osseoperception mediate by anchorage prostheses, their consequential

interactions with the amputee's body and their mediation with the outside world, to improve the process of adjustment and the individual support to each patient.

Part 2 - The role of Osseointegration in bionic prostheses of the upper limb

Subject

Amputation is a demolitive treatment performed in urgency or even in an emergency. It can occur secondary to multiple conditions, including trauma, tumors, infection, peripheral vascular disease, and war-related damages which prevalently involve young and highly functioning people (*1*).

The functional recovery of the amputated limb depends largely on the surgical management of the amputation. Traditional techniques are characterized by principles that are not changed in the last two centuries: preserving the maximum length of the limb, neurectomy, and soft tissue configuration to create padding for the prosthetic socket (*2*). The philosophy behind the traditional technique is to create an ideal accommodation for the stump on the socket, conceiving the stump as passive support for the prosthesis. This led to great difficulty in the aim to create a stump capable to control an advanced robotic prosthesis. Given the progresses of the new generation prostheses that provide an increased number of functions that the amputee must be able to control, the revision of the stump acquires a new and further importance (*3*). Indeed, the recent developments of new control strategies and prosthetic components aimed not only at the functional replacement of the lost upper limb, but also at its integration into the patient's body scheme (embodiment) (*4*). The major revolution in the field of amputation is represented by osseointegration. The Associate Professor Rickard Brånemark is the pioneer of this new surgical approach, realizing the Osseointegrated Prosthesis for the Rehabilitation of Amputees (OPRA) protocol (*10, 21, 35, 37*).

Osseointegrated prosthesis is a special titanium implant characterized by a transcutaneous abutment prosthetic surgically inserted into the bone to make possible prosthesis fixation (20). Ideally, it should provide a stable connection, which allows for physiological weight-bearing and unrestricted range of motion in the adjacent joint. Sockets are still the standard of care in upper and lower limb prosthetics, although they exhibit various shortcomings. Contact between skin and socket may lead to skin irritation and ulceration, as well as increased sweating. This also substantially interferes with interface reliability (*68, 69*).

In the case of short stumps, socket fitting often requires additional fixation, limiting the range of motion in the adjacent joint. Attachment issues, as well as problems with interface reliability, contribute to the high rate of prosthesis abandonment (*1*). OI device should permit overcoming all these issues.

Initially designed for lower limb amputations, osseointegration was first performed in a young woman with bi-lateral transfemoral amputation in 1990 (*28, 35*). Since then, it has increasingly become an established procedure, with standardized protocols for surgery and rehabilitation. It has been employed in transhumeral, transradial, transfemoral, transtibial, and various hand and finger amputations. Good functional outcomes and high patient satisfaction have been reported, with only a few adverse effects. Possible complications include implant failure due to loosening of bone fixation and bone fracture during implantation. Furthermore, the transcutaneous implant is a predisposing factor for stump infection. However, despite frequent colonization by pathogenic bacteria, only a few infections leading to major adverse events such as implant removal, have been described (*32*) (*45*) (*85*). Thus, as underlined before, a secure attachment to the remaining limb is achieved, which results in significantly improved stability in comparison to conventional sockets and socket-mediated skin problems are prevented. Over 400 patients have been treated with the OPRA Implant System in different countries in Europe, Australia, and South America. The OPRA Implant System is a CE-marked medical device.

One of the major successes of osseointegrated implants would seem to be a greater ability of the amputee to live with the prosthetic limb, compared to a suspended alveolar prosthesis. The premise of this innovation arises from a new revolutionary concept named osseoperception. The osseoperception is defined as the improved ability to distinguish vibratory sensations of the prostheses perceived via the implant directly through the bone (1). Probably the loads connected directly to bone thanks to the osseointegration process improve the sensory feedback that should help the control of artificial limb (osseoperceptive sensory feedback) (*21*) 70 64 71). Despite the underlying mechanism of the phenomenon of osseoperception being a matter of debate, patients with bone-anchored amputation prostheses can experience sensory perception, reporting that the prosthesis feels more like their own limb. The physiological integration of implants appears to be of great importance for the patients to provide a more natural function. Osseoperception is vital for both improved prosthetic function as well as for reducing the feeling of being disabled for individuals treated with OI prostheses.

Despite the touch has been considered the main sense for osseoperception mediation, several studies have shown as osseoperception is a multisensory perception, based on activation of the central nervous system (*72*). This point is essential to justify amputees' improved environment perception

with the OI devices and their embodiment process. Embodiment is the perception that something not originally belonging to the self becomes part of the body (*73, 74*) . Already today, it is possible to state that osseointegration and osseoperception improve prosthetic control, prostheses perception and adaptation supporting the creation of a new reinforced image of themselves in which the external device became part of their body (*11*). Bodily incorporation of the prosthesis has significant repercussions on the social and psychosocial adjustments of the patients themselves, giving functional improvements and reserving existential implications in the concept of quality of life (*76, 77*) .
Based on these premises and starting from the hypothesis that the bone-anchored prosthesis improves the quality of amputees' lives mediating sensitive feedback and visual and cognitive impact of own-body, our case study aims to assess the role of osseoperception in the embodiment process.

Materials and Methods

Patient's selection

The patient selected was a 35-year-old man with transhumeral amputation due to polytrauma following a road accident in 2007 (Figure 7). The patient reported having had mild phantom limb symptoms in the days immediately following the amputation surgery, however he had not taken drug therapy. At the time of the interviews, he was wearing an aesthetic prosthesis and a traditional myoelectric prosthesis. He reported that he was not satisfied with the use of both as the aesthetic prosthesis did not allow him to be independent, while he found the control of the myoelectric prosthesis that he only used sporadically to be excessively complex and impractical.

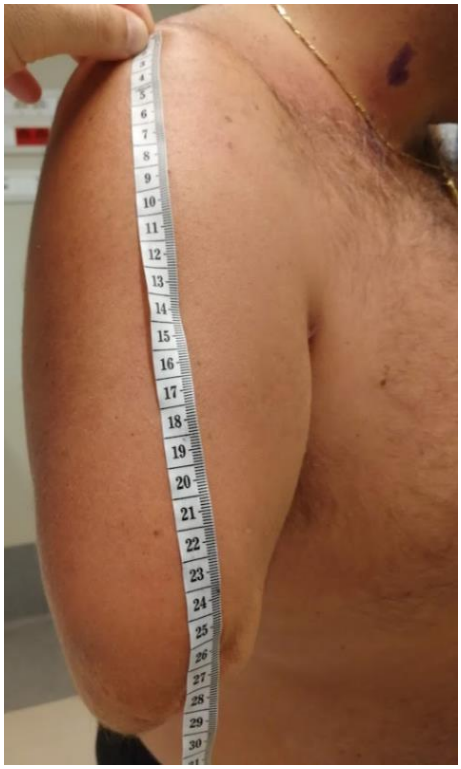

Figure 7: Patient with transhumeral amputation

Operative and post-operative procedures

The surgical intervention consisted in a combination of three procedures: osseointegration (Figure 8), TMR and an innovative TSR.

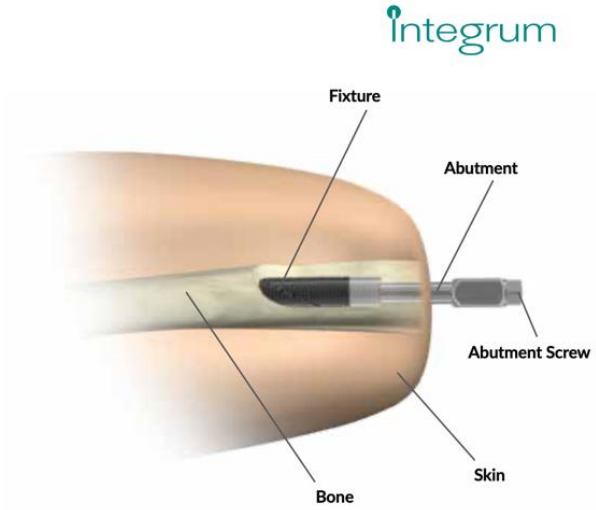

Figure 8: Integrum osseintegrated system : OPRA

Firstly, this reduced the surgical impact on the patient, by carrying out in a single time instead of three (1 for TMR and 2 for osseointegration).

In addition, a further and significant innovation was carried out: with the aim of recreating an area of exclusive sensitivity for the thumb on the amputation stump, the sensory fascicle for the thumb of the median nerve was transposed to the medial cutaneous nerve of the forearm. The operation was performed on 11/09/2019, at the Campus Bio-Medico Polyclinic in Rome, also with the collaboration of Prof. Aszmann.

The surgery consisted in three steps.

- First step: under local anesthesia, medial access was made to the neurovascular bundle of the arm at the bicipital groove, the median nerve was explored, a nerve fascicle was isolated (Figure 14), stimulated by a neurostimulator, the patient reported sensation to the thumb.

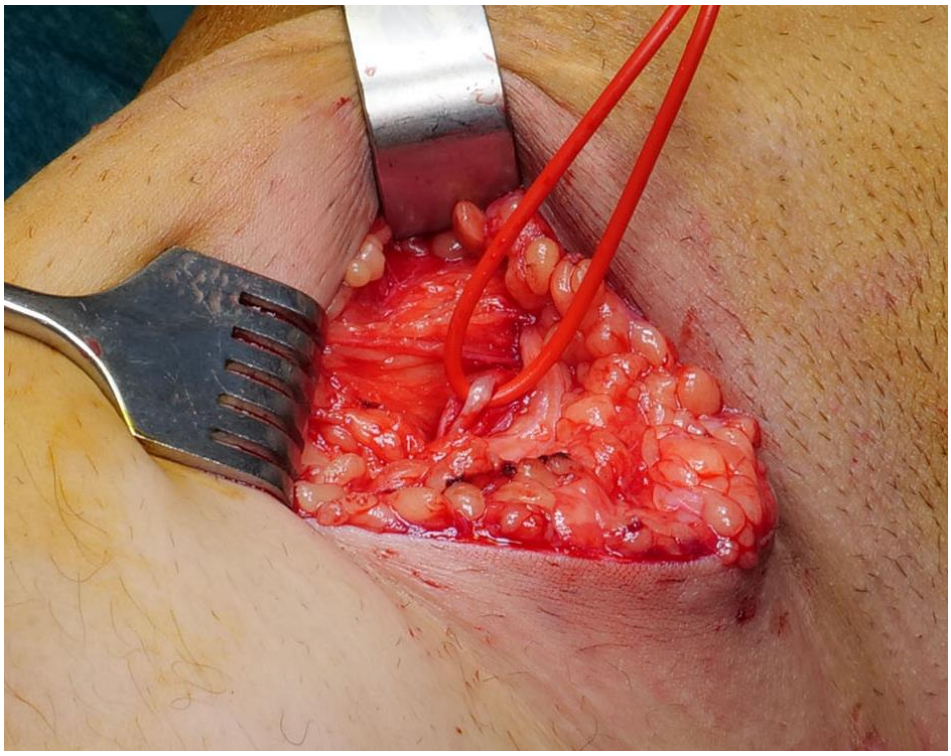

Figure 9: fascicle of the median nerve for sensation of the thumb

- Second step: Osseointegration. Under general anesthesia. Skin incision centered on the previous surgical scar. Exposure of the distal stump of the humerus. An osteotomy of approximately 25 mm is performed due to poor bone quality. The medullary canal is opened with progressive drills up to the size of 13 mm. Subsequently, reaming is carried out up to the measurement of 14 mm (Figure 9). (this corresponds to the first stage of osseointegration "traditional" technique). The control is carried out in fluoroscopy and therefore the positioning of the Fixture (Integrum, Sweden) measuring 14x60mm is carried out and its correct positioning is verified by checking with an ampliscope.

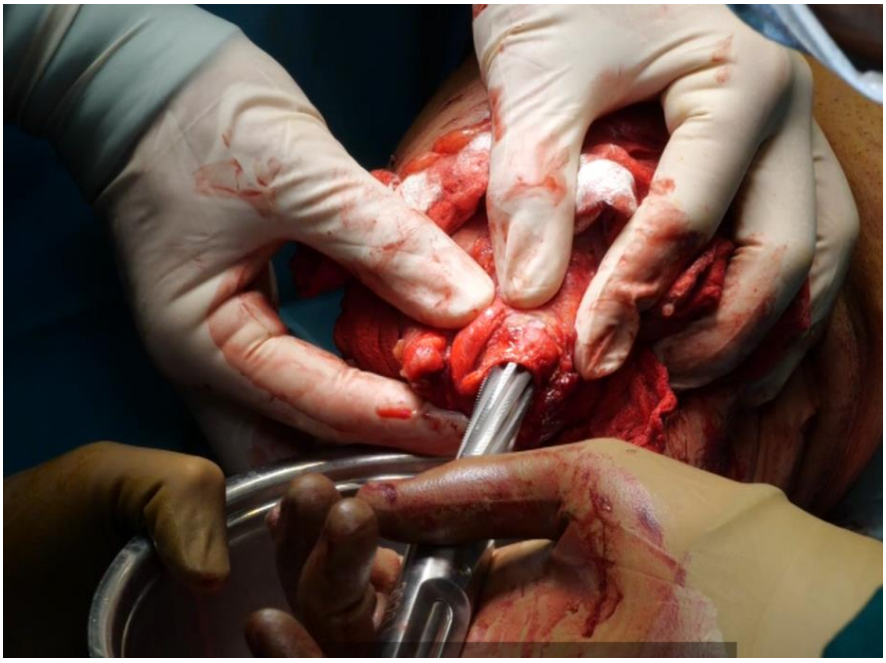

Figure 10: First stage osseointegration: Implant of the fixture

A percutaneous portal with a diameter of about 5 mm is performed on the skin flap and the surrounding adipose tissue is removed to allow the flap to adhere to the bone. Furthermore, the muscle residues adjacent to the periosteum are sutured with Vycril 2.0 thread. Therefore, the thinned flap is positioned in direct contact with the bone, avoiding the interposition of soft tissues. Finally, the abutment is positioned and fixed to the fixture with a screw tightened to 80 N with special instruments. (figure 10)

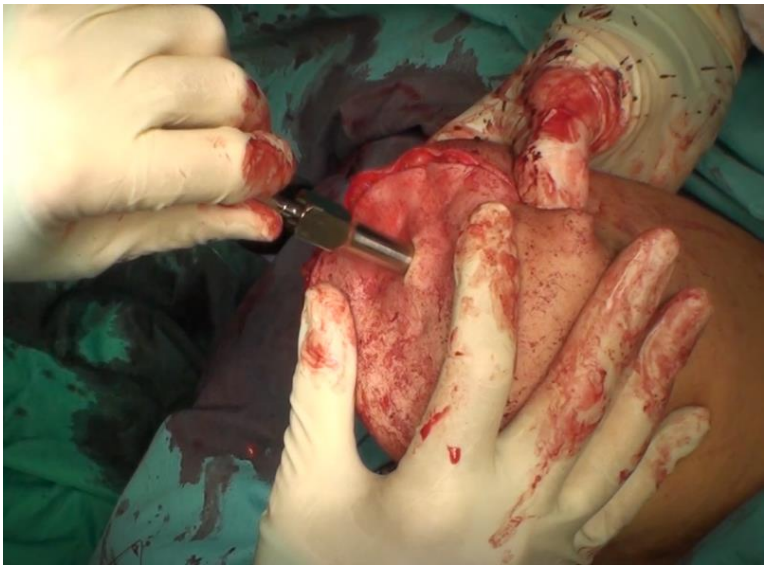

Figure 11: Second stage osseointegration: percutaneous positioning of the abutment and soft tissue plasty

- Third time: TMR and TSR. The previous incision on the medial aspect of the arm was enlarged to extensively expose the median nerve and its previously isolated thumb fascicle. Exposure of the ulnar nerve and musculocutaneous nerve was performed. The musculocutaneous nerve was explored up to its trifurcation, identifying the motor branch for the short head and the motor branch for the brachial muscle. Isolation of the medial cutaneous nerve of the forearm. (figure 11). We therefore proceeded to the section of the aforementioned motor branches and the following nerve transfers was carried out: Median nerve to the motor branch for the brachial muscle, perineural suture performed with Prolene 8.0 thread; Ulnar nerve at the entry point of the motor branch of the short head of the biceps, perineurium-epimysial suture performed with nylon 6.0 thread (Figure 12).

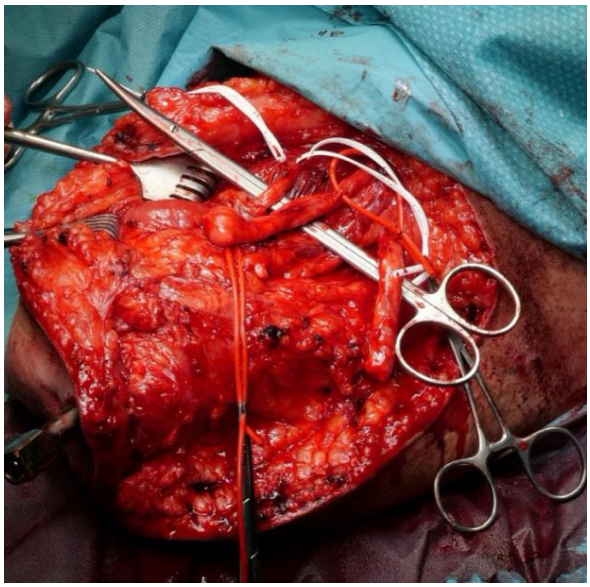

Figure 12: TMR: anterior aspect

Median nerve fascicle for the thumb to the medial cutaneous nerve of the forearm, perineural suture performed with 8.0 prolene thread.

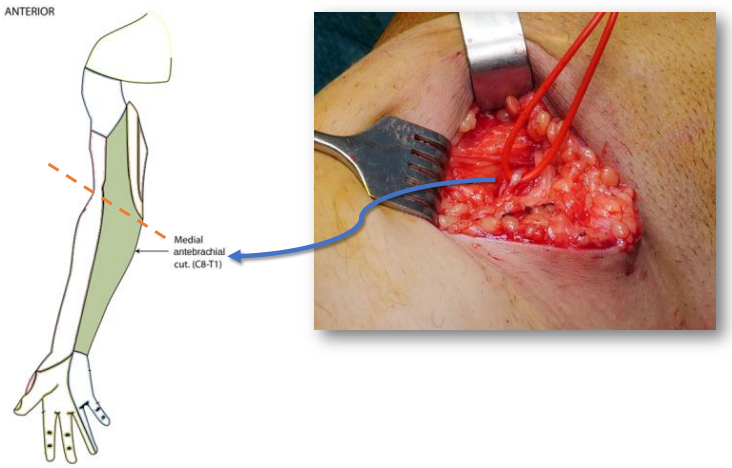

Figure 13: Scheme for innovative TSR

Additional incision was performed along the posterior margin of the deltoid muscle to expose the radial nerve. In the most distal portion, using neurostimulation, it was isolated the motor branches for the brachio-radial muscle, for the lateral head of the triceps and the deep branch of the radial nerve, not responsive to stimuli. This deep branch is in turn divided into two fascicles and the following transfers are made: Medial fasciculus of the deep branch of the radial nerve to the fascicle for the lateral head of the triceps muscle, perineural suture performed using a prolene 8.0 thread; Lateral fascicle of the deep branch of the radial nerve to the fasciculus for the brachio-radial muscle, perineural suture performed using a 8.0 nylon wire. (figure 13)

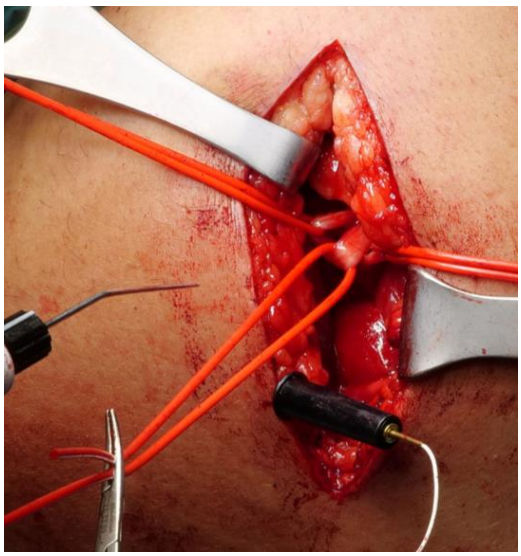

Figure 14: TMR posterior aspect

The patient had a regular post-operative course. He underwent first-line drug therapy (paracetamol + codeine, pregabalin) for the control of phantom limb pain, which was gradually suspended over a period of about 4 weeks, with an excellent clinical response. Wound healing occurred regularly without any complications or delays (post-operative clinical and radiographic images are shown, Figure 14). The reinnervation process was monitored as in the first patient. The osseointegration process was monitored through serial radiographs (1 month, 2 months, 3 months, 6 months, 12 months). (Figure 15)

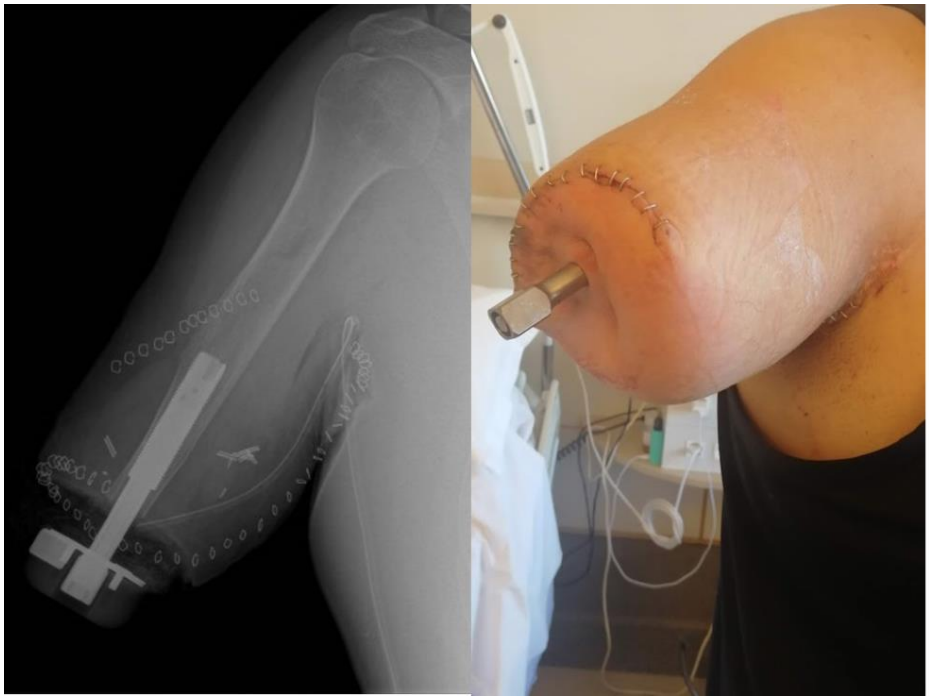

Figure 15: X-ray (left) and postoperative wound (right)

Rehabilitation

The standard osseointegration surgery consists in two surgical times spaced approximately 6 months apart.

In this case, we united the two surgeries in one (OI and TMR-TSR), therefore an *ad hoc* rehabilitation program has been designed for this patient.

Osteointegration training

At month 3 postoperatively, after a short period of training in the presence of doctors, physiotherapists and engineers, the patient was provided with a special kit to manage the exercises independently at home. This choice was also linked to the need to reduce patient travel and visits following the worsening of the frequency of covid-19 infections. The patient was then remote monitored weekly to verify the correct execution of the training and to detect any complications. The kit is constituted by a set of progressive weight bearing provided by Integrum company (Integrum, Sweden). The

rehabilitation protocol consist of progressive weight attachment on the abutment. The patient was asked to apply weights to the implant for approximately 4 hours per day (2 hours in the morning and 2 hours in the evening), starting with 50g and increasing weekly by 50g to around 1300g before being able to hook the prosthesis. In addition to this task, the patient was asked to perform a series of repetitive exercises (10 repetitions) in which, while seated, he had to press the implant on a dynamometer (balance) while maintaining a certain required force applied for a specific time. With the passing of the weeks both the weight value to be maintained and the maintenance time increased: the weight from 5 to 10 kg, the time from 10 to 20 s. The rehabilitation started after the second X-ray taken on 2 months postoperative and continued up to the weight of 1 kg. Subsequently, the patient continued to wear a weight of 1 kg until the application of the cosmetic prosthesis which was provided to the patient at one year after surgery.

In Table 4 are summarized the rehabilitation sessions.

Table 4: Rehabilitation sessions

Week	Bearing on weight scale (kg)	Pression time	Repetitions	Pain, sensations
1	5 kg	10 s	10	No pain
2	6 kg	20 s	10	No pain
3	7 kg	20s	10	No pain
4	8 kg	20s	10	No pain, phantom hand "pulsation"
5	9 kg	20s	10	No pain, phantom hand "pulsation"
6	10 kg	20s	10	No pain
7	10 kg	20s	10	No pain
8	10 kg	20s	10	No pain
9	10 kg	20s	10	No pain
10	10 kg	20s	10	No pain
11	10 kg	20s	10	No pain
12	10 kg	20s	10	No pain
13	10 kg	20 s	10	No pain
14	10 kg	20s	10	No pain
15	10 kg	20s	10	No pain
16	10 kg	20s	10	No pain

Monitoring of the reinnervation process and prosthetic training

At 9th month postoperative, the reinnervation sites of the hand, wrist and elbow were identified at the level of the stump.

These sites useful for the subsequent control of the prosthesis were identified after a somatotopic mapping of the hand-wrist-elbow areas at the level of the re-innervated stump, which therefore allowed to identify the areas of the stump that contracted following the request for moving specific

portions of the phantom limb. These sites were verified and monitored by surface EMG sensors (MyoBock electrode, Ottobock). After identifying the activation thresholds, the patient was asked to perform simple exercises to gain confidence with the muscles to be activated for future control of the prosthesis. It was therefore possible to proceed with training for the control of the prosthesis through the use of a virtual reality system designed within the project, similar to that used for the first patient. Furthermore, in order to reach a stable prosthesis control system as soon as possible, it was decided to provide the patient with training to follow at home. This decision was also made in light of the fewer trips possible due to the COVID19 emergency and, therefore, of the reduced possibility for the patient to reach the Hospital to carry out the training. The patient was trained in home management of the rehabilitation program. He was instructed in the correct positioning of the EMG electrodes at the stump level, in carrying out the home exercise program (which was similar to the one performed in the presence) and in the relative recording of the signal through a specific software (Figure 16) installed on his PC.

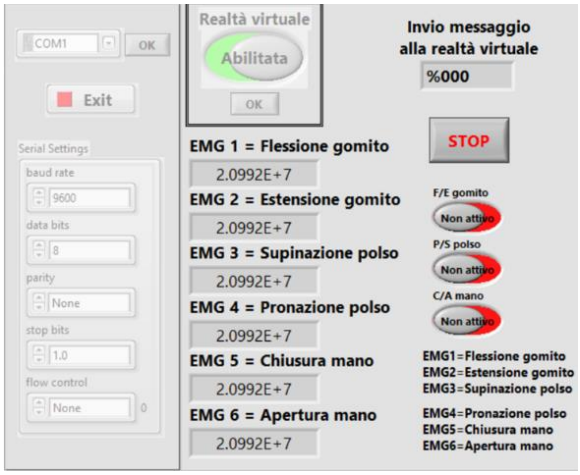

Figure 16: training platform installed on patient's PC. The language is italian

Evaluation of cerebral neuroplasticity and "embodiment" of the prosthesis

Understanding the mechanisms of adaptation of the sensory and motor areas resulting from the intervention can have important implications in the implementation of prosthetic control strategies. Temporal Order Judgment (*86*) and Top Bottom Hand Reaction Time (*4*, *87*) were performed with the aim of understand whether osseointegration may have influenced the body representation of the limb.

Temporal Order Judgment (TOJ)

Temporal Order Judgment (TOJ) is a task in which the subject is required to discriminate the order in which he received two tactile stimuli on a body district. In the present case, electrical stimuli were administered on the healthy hand and on the stump of the subject, when he had his hands parallel or crossed with each other, with the aim of comparing this capacity of the amputee patient with that of healthy subjects (Di Pino et al., 2020) (*4*). Typically, in the healthy population, when the arms are crossed, the accuracy decreases. This phenomenon is called crossing effect. In the upper limb amputees, normally, we cannot observe this crossing effect (Yamamoto and Kitazawa 2001) (*86*). The participant was seated in front of a table, with both her upper limbs lying on its surface in a prone position. Two tactile stimuli were delivered rapidly, one to each limb, with one of the following randomly assigned stimulus onset asynchronies (SOA): -200, -90, -55, -30, -15, 15, 30, 55, 90, 200 ms. Negative intervals indicate that the right limb was stimulated before the left limb and vice versa. The patient had to discern in which limb the first or the second stimulus was delivered. The task was performed either with uncrossed or with crossed arms (Figure 17).

Figure 17: TOJ test

Each experimental condition was tested with eight experimental blocks, four while the subject's arms were uncrossed (with a gap of 40 cm between her hands) and the other four when her arms were crossed. In the crossed conditions, in half of these blocks, the right limb was kept over the left limb and in the other half, the left limb was kept over the right limb. In our experimental blocks, the

participant was asked to verbally report whether the first of the two stimuli were administered on the right or the left limb, while in the other four, on the contrary, he had to report where the second stimulus occurred.

Testing each condition, consisting in a total of 200 trials (20 repetitions per SOA) for the uncrossed limbs and the same number of trials for the crossed limbs, lasted approximately 35 min. In particular, the TOJ test was performed in five different conditions. At 3 months we performed the TOJ test without weight, with only the abutment and with 0.8 kg. At 9 months postoperatively with 1.3 kg and at 18 months with aesthetic bone-anchored prosthesis with and without weight. Such prosthesis was provided at 8 months after the surgery. The weight was added to the prosthesis to achieve the same weight of 1.3 kg of the previous session to explore better the role of visual stimuli.

The order judgment of the subject in each condition was plotted with the different "stimulus onset asynchrony (SOA)" as independent variable (x-axis) and "the probability to judge the right limb as the one firstly stimulated" as the dependent variable (y-axis). Then, data distribution was fitted with a psychophysics sigmoid function:

$$P(SOA, PSS, EA) = \frac{1}{1 + exp\left(-\frac{SOA - PSS}{0.5 \times EA}\right)}$$

Where the two parameters PSS and EA represent:

Point of subjective simultaneity (PSS):

$$PSS = SoA|p_{0.5}$$

This is the SOA value on the curve where the first stimulus had the same probability (p = 0.5) to be felt on the right and on the left limb. The "PSS" indicates how centered is the body pattern of the patient or if there is a lateralization towards the right or left side. It testifies the laterality stimulation bias measured in milliseconds.

Esteem accuracy (EA):

$$EA = \left(2 \times \frac{dP}{dSOA}|SOA = PSS\right)^{-1}$$

This is the SOA needed for the line tangent to the curve at (p = 0.5) to reach the value p = 1. It is the inverse of the slope of the curve multiplied by 0.5. The shorter it is, the more accurate the esteem. Fitting TOJ data with the previous function gives back a value of PSS and EA for each tested condition.

We performed three different analyses.

In the first one, we compared the PSS and the EA resulting from each setup of our amputee and the control group, made of 7 trans-humeral amputees who did not receive the osseointegration surgical technique, by means of Crawford t-tests. The Crawford t-test, instead of comparing the performance with that of a large population with a normal distribution, matches the participant's score against a relatively small control group (i.e., frequently $N < 10$ and typically up to 30). The control group must have done exactly the same task of the single case participant, then a Student-t test was adopted for matching the participant's performance. Under simulations, the method proved to reliably keep under control the alpha error probability to the nominal value of 0.05 (Crawford and Garthwaite, 2007; Crawford et al., 2010).

For the last two analyses, we calculated the EA and PSS fitting values.

In the second analysis we compared these ones between the baseline (the session with no weight) and all the other ones (with weights). We used the Student-t test.

Finally, in the third analyses we evaluated the EA and PSS fitting values between crossed and uncrossed arms of our patient. We used the Student-t test to compare between crossed and uncrossed arms condition in each session.

Top Bottom Hand Reaction Time

In the Top Bottom Hand Reaction Time the participant were seated in front of a desk maintaining a specific posture. (Figure 18)

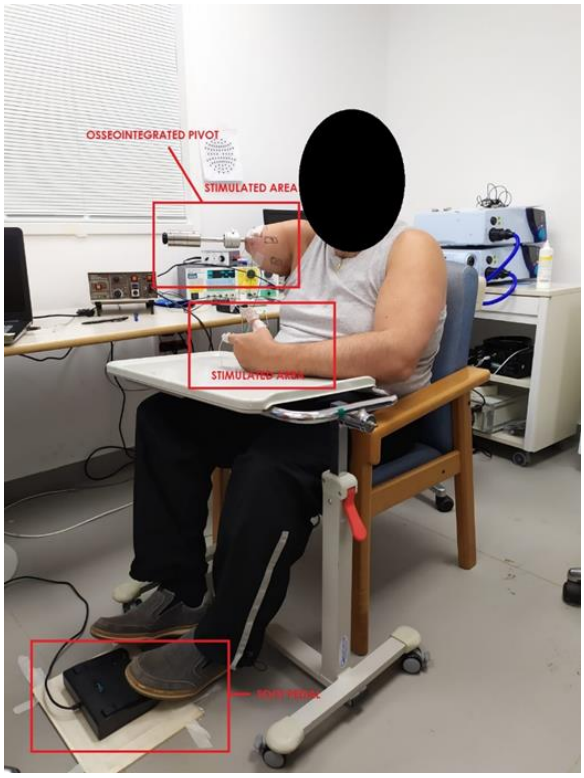

Figure 18: Tactile discrimination trial setup

During the trials, the thumb or index finger of the hands (healthy and phantom) were stimulated in a randomized order while they were positioned at the bottom or top. The patient was asked to report where the stimulation took place (top / bottom) via a pedal connected to a computer that recorded the reaction times. This aspect therefore indicates that there is a fixed posture "integrated" in the body representation. We applied the electrodes on the skin surface where the subjects feel the thumb and index. During the test the patient can step a pedal on the left or right side when the finger placed in top or bottom space is stimulated, respectively. The stimulation is carried out one hand at a time. Typically, in healthy participants, faster responses are associated to the condition of thumb in bottom space and index finger in top space. The Top – bottom reaction time test was performed in five different conditions too: before surgery, at 3 months without weight and with 0.8 kg. At 9 months postoperatively with 1.3 kg and at 18 months with aesthetic prosthesis with weight and a session without any type of weight to understand whether these changes resulted from adding the weight or not. The time of response for the different place of stimulation (stimulated side and finger), position of the arm and session were collected. The ANOVA has been employed to compare each single factor analysed: stimulated finger, space of stimulated finger, hand position and their interactions.

Results

TOJ

The results of TOJ test in the five different setups are represented in Figure 18. On the abscissa there is represented the ISI or interstimulus while on the ordinate the probability, on the left side with the sign -, to feel first the right side that is the amputated one (Figure 19).

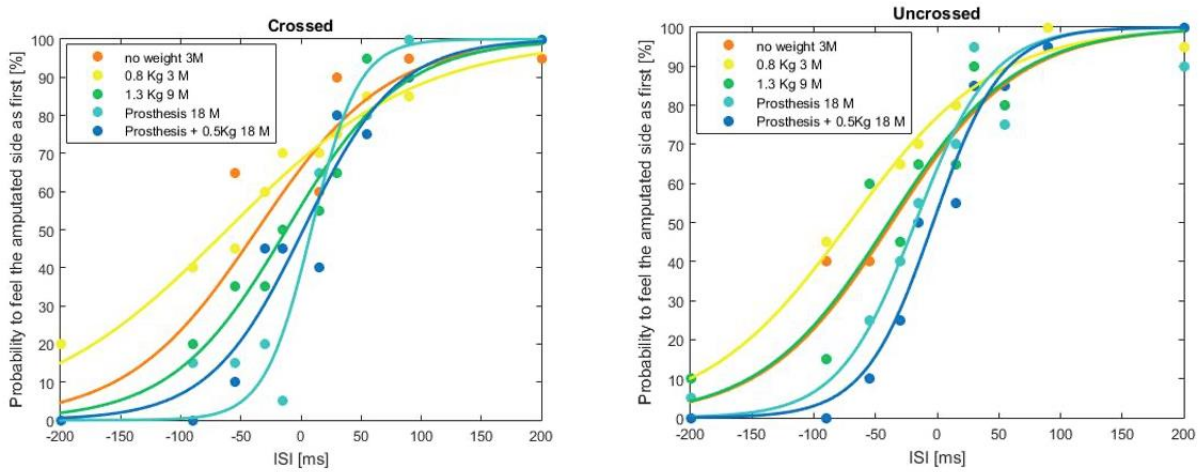

Figure 19: TOJ results of the % clinical sessions

The results of the first analysis have shown that none of the EA and PSS values was statistically significant in all practiced setups, except for PSS of the test made with no weight in crossed arms ($p = 0.04$), and with 0.8 kg both crossed and uncrossed arms ($p = 0.005$; $p = 0.013$, respectively) in the first three months. (Table 5).

PSS		
	UNCROSSED	CROSSED
No weight	$t(6) = 1.76$; $p = 0.128$	$t(6) = 2.59$; $p = 0.041$
800 gr	$t(6) = 3.45$; $p = 0.014$	$t(6) = 4.30$; $p = 0.005$
1300 gr	$t(6) = 1.89$; $p = 0.107$	$t(6) = 0.93$; $p = 0.388$
Aestetic p. No W	$t(6) = 0.82$; $p = 0.442$	$t(6) = -0.48$; $p = 0.649$

Aestetic p. With W	t(6) = 0.11; p = 0.915	t(6) = -0.02; p = 0.984

EA		
	UNCROSSED	CROSSED
No weight	t(6) = -0.01; p = 0.996	t(6) = -0.21; p = 0.841
800 gr	t(6) = -0.16; p = 0.877	t(6) = -0.87; p = 0.419
1300 gr	t(6) = -0.01; p = 0.998	t(6) = -0.05; p = 0.963
Aestetic p. No W	t(6) = 0.45; p = 0.669	t(6) = 0.67; p = 0.527
Aestetic p. With W	t(6) = -0.52; p = 0.624	t(6) = 0.17; p = 0.867

	No weight 3 Months	**800 g 3 Months**	**1300 g 9 Months**	**EA P NO W 18 Months**	**EA P W 18 Months**
PSS (uncrossed) [ms]	-37	-71	-39	-18	-3
PSS (crossed) [ms]	-36	-61	-12	9	2
EA (uncrossed) [ms]	104	118	103	62	56
EA (crossed) [ms]	108	160	96	39	78

Table 5: TOJ First analysis

From the second analysis we noticed that, except for the 800g session, where both the PSS and EA get worse respect to baseline, all PSS and EA values improved in all sessions compared to the baseline. This improvement was most evident in the sessions with the cosmetic prosthesis (Table 6).

PSS

	UNCROSSED	CROSSED
No w. vs 800 gr	$t(16) = 12.35$; $p < 0.001$	$t(16) = 6.69$; $p < 0.001$
No w. vs 1300 gr	$t(16) = 0.66$; $p = 0.521$	$T(16) = -5.89$; $p < 0.001$
No w. vs Aesthetic p. No W	$t(16) = -5.12$; $p < 0.001$	$t(16) = -9.43$; $p < 0.001$
No w. vs Aesthetic p. With W	$t(16) = -10.35$; $p < 0.001$	$t(16) = -8.05$; $p < 0.001$

EA		
	UNCROSSED	CROSSED
No w. vs 800 gr	$t(16) = -5.06$; $p < 0.001$	$t(16) = 13.79$; $p < 0.001$
No w. vs 1300 gr	$t(16) = 0.06$; $p = 0.956$	$t(16) = 3.08$; $p = 0.0072$
No w. vs Aesthetic p. No W	$t(16) = 11.11$; $p < 0.001$	$t(16) = 12.35$; $p < 0.001$
No w. vs Aesthetic p. With W	$t(16) = 14.73$; $p < 0.001$	$t(16) = 14.52$; $p < 0.001$

	No weight 3 Months	800 g 3 Months	1300 g 9 Months	EA P NO W 18 Months	EA P W 18 Months
PSS (uncrossed) [ms]	-37	-71	-39	-18	-3
PSS (crossed) [ms]	-36	-61	-12	9	2
EA (uncrossed) [ms]	104	118	103	62	56
EA (crossed) [ms]	108	160	96	39	78

Table 6: TOJ second analysis

The third analysis showed the improving of the EA values in all sessions, except for the 0.8 kg setup. On the other hand, the PSS values became more positive in all sections, except that with 0.8 g, reaching the maximum value in the sections with the cosmetic prosthesis. Finally, we observed that the crossing effect (worsening of the accuracy for crossed condition with respect to the uncrossed one evaluable with EA uncrossed values smaller than EA crossed values) occurred only during the 0.8 g sessions ($p < 0.001$) and when the weight was applied to the cosmetic prosthesis ($p < 0.001$) (Table 7).

PSS	
No weight	$t(16) = 0.11$; $p = 0.912$
800 gr	$t(16) = 5.54$; $p < 0.001$
1300 gr	$t(16) = 7.35$; $p < 0.001$
Aesthetic p. No W	$t(16) = 6.29$; $p < 0.001$
Aesthetic p. With W	$t(16) = 7.35$; $p = 0.183$

EA	
No weight	$t(16) = 1.10$; $p = 0.29$
800 gr	$t(16) = 24.13$; $p < 0.001$
1300 gr	$t(16) = -2.07$; $p = 0.055$

Aesthetic p. No W	t(16) = -5.49; p < 0.001
Aesthetic p. With W	t(16) = 5.96; p < 0.001

	No weight 3 Months	**800 g 3 Months**	**1300 g 9 Months**	**EA P NO W 18 Months**	**EA P W 18 Months**
PSS (uncrossed) [ms]	-37	-71	-39	-18	-3
PSS (crossed) [ms]	-36	-61	-12	9	2
EA (uncrossed) [ms]	104	118	103	62	56
EA (crossed) [ms]	108	160	96	39	78

Table 7: TOJ third analysis

Top Bottom Hand Reaction Time

During the experiment, we noticed that the reaction time decreased throughout the four sessions for both limbs (Figures 20- 23), with lower values when the amputated arm was placed up (Figure 24). The contrary occurred in the healthy side. During the 18 months postoperatively, the reaction times were lower in the session with cosmetic prosthesis and weight, especially for the amputated side, compared to the session without weight. These results have not showed statistically significance ($F(1,24) = 1.82$, $p = 0.190$).

The last analysis, performed on the evaluation of each factors evaluated (finger stimulated, space of stimulated finger, hand position) and theirs interactions (Finger Stimulated/Space of Finger Stimulated; Finger Stimulated/Hand Position; Space of Finger Stimulated/Hand Position; Finger Stimulated/Space of Finger Stimulated/Hand Position), showed statistically significative differences between the sessions regarding the finger ($F(1,24) = 7.99$, $p= 0.009$) and the interaction between the stimulated finger space and the hand position ($F(1,24) = 57.25$, $p < 0.001$). (Figure 25)

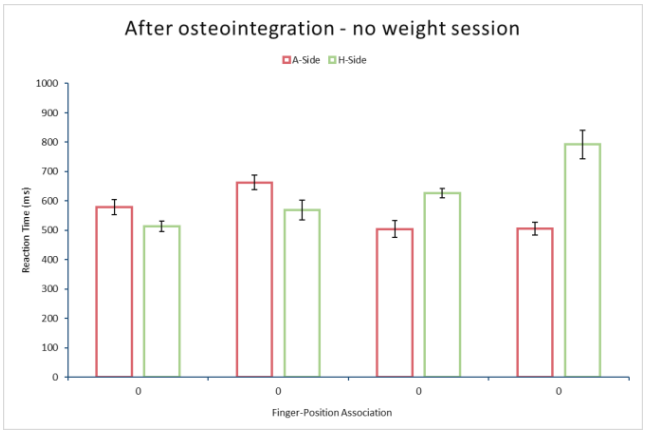

Figure 20: TOJ results of the % clinical sessions

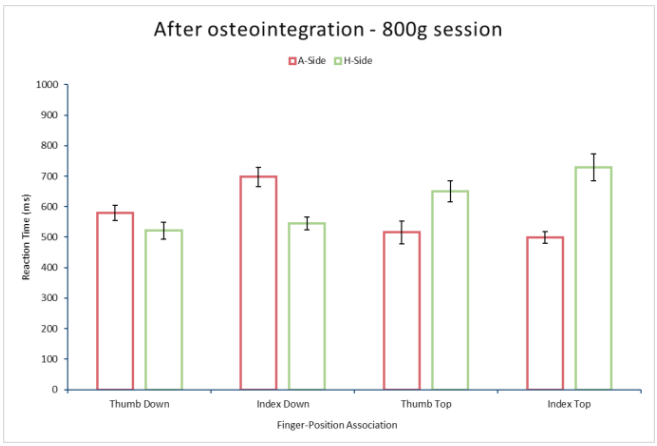

Figure 21: Top Bottom Hand Reaction time 800gr

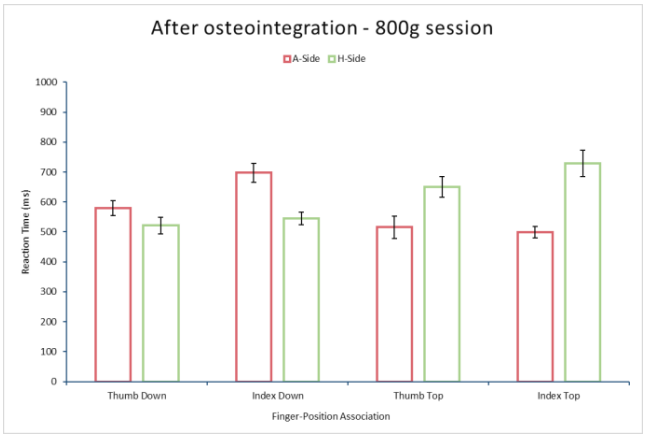

Figure 22: Top Bottom Hand Reaction Time 1300gr

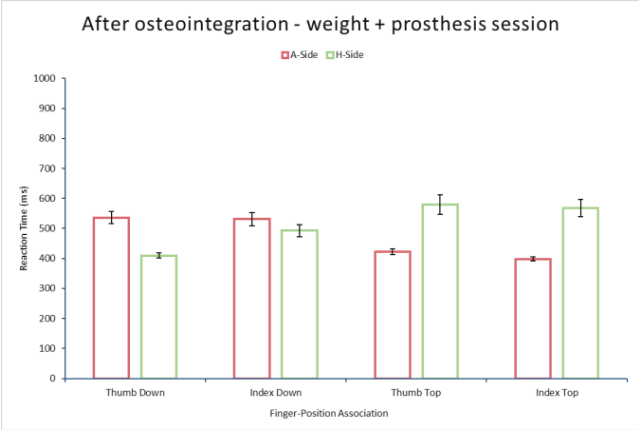

Figure 23: Top Bottom Hand Reaction Time cosmetic prosthesis with weight

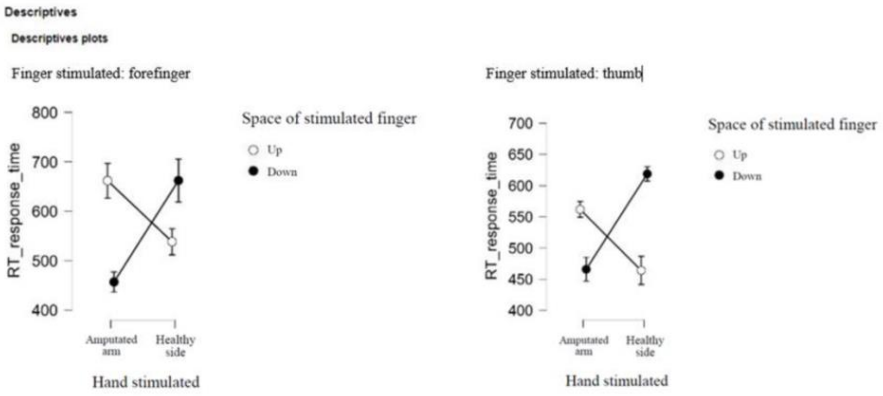

Figure 24: Reaction time finger referred

ANOVA - RT_response_time

Cases	Sum of Squares	df	Mean Square	F	p
Stimulated hand	11146.131	1	11146.131	3.058	0.093
Stimulated finger	29129.767	1	29129.767	7.992	0.009
Space of stimulated finger	66.369	1	66.369	0.018	0.894
Weight	6637.160	1	6637.160	1.821	0.190
Stimulated hand * Stimulated finger	235.920	1	235.920	0.065	0.801
Stimulated hand * Space of stimulated finger	208680.771	1	208680.771	57.252	< .001
Stimulated hand * Weight	4.654	1	4.654	0.001	0.972
Stimulated finger * Space of stimulated finger	10503.151	1	10503.151	2.882	0.103
Stimulated finger * Weight	1907.869	1	1907.869	0.523	0.476
Space of stimulated finger * Weight	2186.118	1	2186.118	0.600	0.446
Stimulated hand * Stimulated finger * Space of stimulated finger	5866.138	1	5866.138	1.609	0.217
Stimulated hand * Stimulated finger * Weight	724.128	1	724.128	0.199	0.660
Stimulated hand * Space of stimulated finger * Weight	1642.674	1	1642.674	0.451	0.508
Stimulated finger * Space of stimulated finger * Weight	821.193	1	821.193	0.225	0.639
Stimulated hand * Stimulated finger * Space of stimulated finger * Weight	6441.702	1	6441.702	1.767	0.196
Residuals	87479.195	24	3644.966		

Note. Type III Sum of Squares

Figure 25: Top Bottom Hand ANOVA analysis results

Discussion

The aim of our study was to assess the role of osseoperception in the embodiment process, assuming that the bone-anchored prostheses play an essential role in the mediation of sensitive feedback and visual and cognitive impact of own-body. We tested patient changes in body representation through two behavioral tests TOJ and the Top Bottom Hand Reaction Time. In the TOJ, the subject was required to discriminate the order in which he received two tactile stimuli on a body district in several different sessions (with and without weight). We conducted three different analyses.

In the first analysis we compared the EA and PSS among the control group (7 trans-humeral amputees) and our patient, highlighting that the are no statistical differences except form the 800 gr session. We hypothesized that this phenomenon is due to a settling phase since we also performed TMR and TSR in the same surgery. Therefore, this adjustment is probably not strictly connected to OI process. In the second analysis we compared the fitting of EA and PSS between the baseline session and the others one, observing that there was an improvement of PSS and EA in all sessions, except for 800 gr session. This improvement was most evident in the sessions with the cosmetic prosthesis. In this sense, the visual stimulus seems to play an important role, but, as we evaluated in the third analysis, it alone is not sufficient to restore the normal body representation. At the same way, the addition of a sensitive feedback through a weight to the anchored prosthesis seems to give a significative contribution to restore the normal body scheme like the healthy subject. Typically, in the healthy population, when the arms are crossed, the accuracy of the perceived stimuli decreases. This phenomenon, called crossing effect, was tested by Yamamoto and Kitazawa (*88*) in their experimental study conducted in 2001. In particular, they tested the TOJ on 20 healthy subjects, observing that subjects could judge temporal order when their arms were crossed, but only if given adequate time (>1 s). At moderately short intervals (<300 ms), crossing the arms provoked misreporting (that was, inverting) of the temporal order. Thus, at these intervals, the determining factor of temporal order was the spatial location of the hands. Consequently, they assessed that it is not until the spatial locations of the hands are considered that the cutaneous signals from the respective hands are ordered in time.

Our results from TOJ are matched by current literature data.

The prosthesis embodiment through the study of multisensory integration and spatial remapping were developed in healthy subjects (as reported in the upper lines in the Yamamoto and Kitazawa, 2001 study), demonstrated to be sensible to the embodiment of tools and were already validated in amputees. Canzonieri et al.(*89*) assessed the multimodal representations of the body (BRs) and of the space around the body (PPS) in a group of upper limb amputees by means of a tactile distance perception task and PPS by means of an audio-tactile interaction task. Subjects performed the tasks

with stimulation either on the healthy limb or the stump of the amputated limb, while wearing or not wearing their prosthesis. When patients performed the tasks on the amputated limb, without the prosthesis, the perception of arm's length shrank, with a concurrent shift of PPS boundaries towards the stump. Conversely, wearing the prosthesis increased the perceived length of the stump and extended the PPS boundaries to include the prosthetic hand, such that the prosthesis partially replaced the missing limb. The most significative confirmation about our data is due by Sato et al (2017)(*90*). They investigated the incorporation of prosthetic arms in amputees employing the crossed hands illusion. Successive somatosensory stimuli are delivered, one to each arm, at intervals of 300ms or less, and where arm crossing often causes inversion of perceived tactile temporal order. The induced reversal illusion was greater with a prosthetic limb than without in three amputees. With a shorter prosthetic arm (i.e., one that did not reach the contralateral limb), the illusion induced by vision of the short prosthetic arm was significantly reduced as compared to that seen when the long prosthetic arm crossed over the other arm. These results therefore suggest that the somatosensory signals were referred to the spatial location of the tips of the prosthetic arm, which was incorporated into the body representation by the amputees. Certainly, osseointegration is not the exclusive factor which influence the embodiment process. In their study, Di Pino et al. (*4*) showed as patient training play an essential role in the mechanism of prosthesis incorporation. They tested the prosthesis embodiment in a longitudinally research in an amputee who had received feedback through intraneural and perineural multichannel electrodes implanted evaluated in her stump. Three factors — invasive (vs non-invasive) stimulation, training, and anthropomorphism — have been tested through multisensory integration which included the crossing-hand effect in temporal order judgment (TOJ), test high sensible to action-oriented remapping. Results from the amputee participant were compared with the ones from healthy controls. In the TOJ with uncrossed limbs, when the right limb was stimulated non-invasively and the left invasively, there was a significant right laterality bias, so that the participant perceived intraneural left stimulation with about 30 ms delay compared to the healthy limb [point of subjective simultaneity (PSS) = + 28.9, CI: (+14.8 ms, +42.5 ms)]. PSS was significantly different than the one of the control group only when the left limb was stimulated invasively [non-invasive/intraneural vs control: $t(10) = 2.582$, $p = 0.027$, Z-CC = 2.69 CI (1.37, 3.98); noninvasive/non-invasive vs control: $t(10) = 0.946$, $p = 0.336$)].

When the left limb was stimulated intraneurally and the right limb non-invasively, the performance of the task was not worse than when both limbs were stimulated non-invasively, despite the asymmetric stimulation. Indeed, both conditions had esteem accuracy (EA) not different than the one of the control group (non-invasive/intraneural vs control: $t = 1.011$, $p = 0.336$; non-invasive/non-invasive vs control: $t = -0.383$, $p = 0.71$). Thus, they obtained a worsening of TOJ performance

following arm crossing when the patient worn the more trained, despite less anthropomorphic, prosthesis, suggesting that training was critical for our participant to achieve operative tool-like embodiment.

In the Top Bottom Hand Reaction Time the patient was seated in front of a floor maintaining a specific posture. During the trials, the thumb or index finger of the hands (healthy and phantom) were stimulated in a randomized order while they were positioned at the bottom or top in five different sessions. The patient was asked to report where the stimulation took place (up / down) via a pedal connected to a computer that recorded the reaction times. This aspect therefore indicates that there is a fixed posture "integrated" in the body representation. The results of these experiments showed that there were statistical differences within the sessions regarding the stimulated finger, the hand position and the interaction between the stimulated finger space and hand position. In the healthy patient the was an interaction between the space of the stimulated finger and the position of the hand. This finding allows to hypothesize that the body pattern varies and reorganizes itself after the amputation procedure, and it changes the frame of reference that moves from the fingers to the whole limb. The prerequisites for understanding the importance of this task in prosthetic-related body reworking derive from previous studies conducted on healthy patients (*91*). Romano et al. showed as body parts have preferential connections with relative spatial sites. They conducted three experiments, in which they found consistent preferential associations between the index finger and the top position, and between the thumb and the bottom position. This association was experimented through a tactile sensory discrimination task, made both with and without vision, as well as at the implicit conceptual association level. These findings demonstrate that body parts and spatial locations are stably linked. Therefore, not only are body segments dynamically mapped in space for perception and action, but they also hold intrinsic spatial information that contributes to somatosensory spatial processing. In the literature, there are no data attributable to the same task performed on amputees. So, this is certainly a huge strength from which to start for further research into body perception in amputees.

Limitations

Our study in based on the analysis conducted on a single patient, with a small simple size of control groups. Moreover, we have analyzed only two behavioral tests with a relative short follow up.

Being a clinical study, the main significant limitation was due to the preliminary data related to the COVID-19 pandemic condition. The monitoring of the body representation through other specific protocols for the evaluation of brain plasticity and body representation is needed to consolidate our results. The last weakness point of the present study is that the patient had never worn the final

myoelectric osseointegrated-prosthesis. Also, this limitation is related to the difficulties due to the pandemic.

Future Directions

As technological progress in the field of robotic prosthetics has made great strides, in recent years surgical techniques have been developed that are aimed at adapting the upper limb amputation stump to the use of increasingly advanced prostheses.

In this scenario, clinicians and surgeons cannot be only, almost blind, final applicators of a ready-to-be-used technology, but they are active actors in designing the best combination of possibilities to obtain the result of adapting the human body to advanced technology, up to the goal of making it a whole one.

Our study confirms as osseointegration represents a consistent advantageous surgical procedure for amputees, more adaptable than the conventional socket prosthesis. OI-prosthesis represents a promising treatment option for carefully selected patients, not only for its improved functional outcomes and higher quality of life but also for its significant role in the incorporation prosthesis process.

The results of our experiments on behavioural tasks demonstrate as the body perception changes, increasing, thanks to the use of this device. Osseointegration undoubtedly contributes to prostheses success, strengthened their acceptance thank to the phenomenon of osseoperception and its essential contribution in the embodiment process. It is essential to underline that the perception of prostheses as an essential and integral part of the body brings radical changes beyond the functional improvements, including existential implications in the concept of quality of life. Furthermore, we must specify as these results could be further improved through the application of new systems of neuromuscular integration that enables volitional motor control of, and proprioceptive and sensory feedback from, the artificial limb, especially in the upper extremity. Among these, the enhanced OPRA (e-OPRA)(*92*) system and the Agonist-antagonist myoneural interfaces (AMIs) (*93*) are the most innovative. The e-OPRA system permits bidirectional communication between implanted neuromuscular electrodes and the external prosthesis thanks to the Osseointegrated Human-Machine Gateway (*45*), tested in trans-humeral amputees. On the other hand, AMIs procedure is based on the connection of muscles with naturally oppositional functions with the aim to emulate native limb dynamics, permitting for voluntary prosthetic control and proprioceptive feedback of joint position (*94*).

Although they are at the beginning of their experimentation, neuromuscular integration associated with osseointegration will permit more natural prosthetic function and improved outcomes, probably contributing to the complex and multisensorial embodiment processes.

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