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Characterization and bioactivation of synthetic absorbable thread sutures for improved healing and cosmetic outcomes

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"… as patients, physicians and scientists,

we must begin to develop a vision of what we want from science and medicine.

The limits of medicine are not technological, they are conceptual,

and all of us together must define the concept"

Edward S. Golub

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Preliminary remark

The format of this dissertation is publication-based according to the "Regulations for the Award of Doctoral Degrees" of the Technical University of Munich, effective January 1st, 2014, appendix 6 [ad §6 (9)] for the Doctoral-Program "Experimental Medicine", of the Medical Graduate Center, Technical University of Munich, as it meets the following criteria:

(1) The dissertation includes an introductory section, with a discussion across dissertation topics including a review of relevant literature
(2) The dissertation is based on publications in international peer-reviewed journals, which are accepted for publication or published. The doctoral candidate is first author of two publications.
(3) The dissertation contains a brief summary of each publication and the doctoral candidate's individual contribution
(4) The format of publication-based dissertation is supported by the Mentor

1. Introduction

1.1 Thread sutures

In order to avoid complications like excessive bleeding, poor healing and infections, acute wounds need proper cleansing and care. According to their severity, such proper care usually implies an approach that enables secure tissue approximation, in the interest of protecting the affected area while promoting better, and faster healing by first intention [1].

A suture is a strand of material to ligate blood vessels or to approximate tissues together. Sutures are the standard of care for wound approximation and have been at the forefront of surgical medicine throughout time [2-4].

Archeological records dating from more than 30,000 years ago [5, 6] show the usage of multiple materials like hair, bristles, grass, cotton, linen, bark fibers, silk, animal gut, ant heads, and sinew as means to approximate tissues [7, 8]. Along with these, non-biological materials have also been used for holding tissues together, like iron and steel strings [9]. Nowadays, those materials have been replaced by newer, better characterized, and standardized synthetic sutures, while, new others keep emerging, *e.g*. sutures made of polymers produced by genetically engineered organisms such as spiders and *E. coli* strains [10].

There are many methods for mechanical wound closure such as staples, tape, and adhesives [7, 11, 12], yet, they fail to provide the stability and flexibility rendered by sutures in wound management [13]. Therefore, sutures remain the most widely used materials in wound closure [14].

Suturing seek to maintain wound closure until the tissues are strong enough to withstand daily tensile forces, and to enhance wound healing when the wound is most vulnerable. The correct approximation and stabilization of wound margins influence the success of a surgical procedure and the final cosmetic aspect. For this, scientists around the world search for the development of an ideal suture material, whose characteristics actually vary according to the patient and the procedure to perform.

Even though the number of absorbable and non-absorbable sutures available could be considered high, the types of the commonly used sutures at present in clinical practice appear to be limited, considering that, they fulfil many handling, mechanical and safety requirements, but not all. Additionally, improving their versatility and adaptability would be valuable by providing more options for clinicians, while current efforts are centered on developing suture materials with improved physical and mechanical properties along with additional capabilities such as to deliver drugs and cells to improve wound healing [13].

The purpose of this work is to contribute to the improvement of surgical threads by means of evaluating commercially available barbed threads, and developing bioactive photosynthetic sutures with improved regenerative capacities.

1.2 Characteristics of thread sutures

The major function of thread sutures is to bring and hold tissue together following separation by surgery or trauma [7]. The use of sutures looks for obliterating the dead space where the tissues are interrupted by bringing together the epithelial layers, while

distributing and maintaining an appropriate level of tension along the fixing points across the wound. With this, they attain a functional closure, minimizing bleeding and scar formation, and decreasing the risk for infection [7, 14]. Such functions of sutures have a key role in wound closure, offering a significant improvement when compared to similar wounds healing by second intention, or even making a difference between life and death [1, 11, 15].

There are several criteria by which suture threads can be classified, such as

a) origin (natural or synthetic),

b) based on characteristics such as absorbability, or

c) their configuration (monofilament or multifilament, also called braided sutures) [2, 7, 8].

Each one presents advantages and disadvantages to be considered when choosing the material to use. Yet, there are essential characteristics that any suture should fulfill. According to Pillai (2010), ideal threads should display the following characteristics [7]:

1. Sutures should be easy to sterilize.
2. Elicit minimal tissue reaction as well as no allergic reaction nor carcinogenic action (being biologically inert, free of contaminants, not supporting bacterial growth, and neither causing nor promoting tissue adverse reactions or complications).
3. Uniformity, in terms of diameter and size, for providing at the same time uniform and high tensile strength by suture type and size.
4. Proper pliability for ease of handling and knot security.

5. Displaying different degrees of absorption, ideally being absorbed right after serving its function.
6. Being very strong to avoid dehiscence, but also able to dissolve in body fluids and lose strength at the same rate that the tissue gains strength.

In short, sutures should provide a secure wound closure during an adequate amount of time, in order to promote healing by primary intention and to prevent dehiscence [7, 11, 12].

Three pivotal factors determine the outcome when it comes to wounds that need to be sutured: 1) the surgeons' technique and expertise,

2) the patient and his/her physiological state, and

3) the chosen suture itself.

There is no perfect suture suitable for every circumstances and procedures, therefore physicians must choose from the accessible products in the market, and this selection is equally essential as using a proper suturing technique for obtaining good cosmetic results, avoiding scarring and poor wound healing [3, 7, 8]. For this, it is essential to rely on updated information, based on standardized scientific data and a comprehensive responsible analysis.

In clinical practice, intrinsic characteristics of the host tissue and the suture material itself determine the selection of the thread suture. Important aspects to be highlighted include anatomical and physiological factors such as presence of infection, wound location, tension across and on wound edges, age, cosmesis, number of tissue layers involved, depth of suture placement, and expected time of suture removal. Healing rates

of tissues differ depending on factors inherent to each patient such as infection, irrigation problems, obesity, collagen disorders, malnutrition, malignancy, presence of edema, drug consumption (for example cytotoxics and steroids), among others [7, 9, 16-18].

The surgeon should choose the right suture for the type of surgery that he/she is performing based on the requirements of the tissues, and on his/her familiarity with the available products. Concerning the threads, color of the material (easily visible), its availability, and cost are definitely important. Possession of adequate strength, and the proneness to elicit inflammatory reactions should also be considered.

Thread sutures characterize by their physical and mechanical properties, handling characteristics, as well as by their biological and biodegradation behavior.

The mechanical properties include tensile strength, elasticity, memory, plasticity and others. The strength is the most frequently reported mechanical feature of suture materials, which should ideally correlate with the tissue strength [7]. It is defined as the amount of weight (also called breaking load) necessary to break a suture (referred as breaking strength) divided by the cross sectional area of the suture [19]. Therefore, it is dependent on the diameter of the suture, but also, on the material itself.

Elasticity is the inherent ability of the suture to regain its original form and length after having been stretched, while the ability to retain its new deformed length and form is referred to as its plasticity. Many suture materials are elastic, but only a few, are also plastic (such as polypropylene). Elasticity and plasticity become important when there is swelling of a wound. When the tissues swollen, an elastic thread will stretch, not causing further harming, but when the swollenness resolves, sutures with plastic properties will retain its stretched size, and thus its tied loop may be too loose to oppose

the wound edges [9]. Memory relates to both, elasticity and plasticity, indicating a suture's capacity to return to its former shape upon deformation such as knotting [9].

The physical characteristics regulated by authorities include the configuration (single-stranded, monofilamentous or multistranded, multifilamentous), their size (referring to their diameter or caliber), and the capillarity (ability to absorb fluid) [9, 12, 13].

Handling characteristics of surgical threads relate to their pliability, as well as their coefficient of friction. Pliability is determined by both, the suture material and its configuration, providing enough flexibility (or lack of stiffness) to accomplish surgical suturing and knot tying [20]. The friction coefficient of a suture is a measure of the slipperiness of the material, which influences the how much the suture tends to drag through tissue and how much the knots tend to loose [9, 12, 21].

There is very few information available about the frictional behaviors of surgical sutures in sliding contact with tissue and about the friction of surgical sutures during stitching [22]. This led to the development of a new field called “tribology”. Tribology refers to the science and engineering of interacting surfaces in relative motion, considering the principles of friction, lubrication and wear [21, 23].

Tissue interactions are also to take into account when choosing a suture, particularly because different tissues have different healing rates and requirements for suture holding and support, with some needing only a few days (*e.g.*, muscle, subcutaneous tissue, skin), while others require weeks or even months (*e.g.*, fascia and tendon) [7]. Concurrently, such healing rate depends on the metabolic and physiological condition of the patient.

Additionally, as foreign biomaterials, sutures evoke a tissue reaction. High friction between surgical sutures and tissues may aggravate inflammation and pain in the area surrounding the wound [22].

From day two to seven after implantation there is a peak in the inflammatory response against the suture material. This may lead to the softening of the sutured tissue, infection, dehiscence and delayed wound healing, if not handled properly [9]. Allergy to some suture materials has been reported in the medical literature, independently of their natural or synthetic origin [24-26], and may also lead to delayed wound healing [27]. Additionally, severe delayed tissue reactions have been reported to some other biodegradable materials [28].

In the case of non-absorbable sutures, the inflammatory reaction is minimal, but a thin fibrous capsule develops covering the suture material, usually by 28 days post-implantation. With absorbable sutures, the inflammatory reaction is greater and persists until the suture is either absorbed or extruded [9].

Some frequently used absorbable materials include poliglactin 910 (PLGA), polyglecaprone 25 (PGCL) and polydioxanone (PDO). Poliglactin 910 has an absorption rate of 56 to 70 days. PDO is preferred for the treatment of slower-healing wounds due to its longer strength retention, supposed to be of up to 90 days, with an absorption rate in the range of 180 to 210 days [9, 10].

All of the afore mentioned characteristics are important factors interacting with each other and influencing both, the performance of the sutures and the tissue reaction. For instance, braided sutures display higher flexibility and pliability and are therefore usually easier to handle and tie than monofilamentous ones. On the other hand, their use implies an increased risk of infection because of the possibility of harboring

microorganisms into the interstices of the filaments and knots, where they are relatively protected from leukocytes and can therefore induce and sustain infection [9, 13].

In connection to this, characteristics such as capillarity and fluid absorption ability directly affect the tendency of sutures to take up and retain bacteria. Additionally, suture thickness directly relates to the amount of force required to cut through the tissue: a small diameter generates more force per unit area than a suture of the same material with a larger diameter. Stiff, inelastic or high tensile strength sutures have a greater tendency to cut through tissue at the time of implantation, due to excessive tension during placement or afterwards, because of wound swelling or sudden mechanical forces acting on the wound (like when coughing) [9].

For each individual product, the manufacturers provide information that is often decisive for best choosing of the suture [10].

In the recent years, sutures which are not intended to be knotted, have been cleared by the regulatory entities to be used in several surgical applications [29]. For instance, barbed sutures are threads that have sharp projections or barbs on their surface, which allows them to anchor into tissues linearly. This is particularly useful in cases where the presence of knots may have adverse effects on the tissues, or when placing a knot might be difficult, like in deep surgical regions, or minimally invasive surgical procedures [13]. By avoiding the need of knotting, these new sutures provide additional advantages, like reducing the suturing time, the risk of dehiscence, and infection rates, offering as well an improved uniformed distribution of tension along the incision line, and the possibility of improved cosmesis [29]. However, an important disadvantage is that, because of their innate design with barb front, they may puncture the surgeon's glove, carrying a risk for both, the surgeons and the patients [13].

With ever-changing technology and a better understanding of cellular science, suture technology has developed in order to provide better quality and outcomes. This development is not only closely related to scientific progress [10], but also accompanied by an industry driven pull [8]. As example of the latter, suture industry has registered a tremendous growth during the last two decades with sutures becoming the largest group of biomaterials, with a huge market exceeding $1.3 billion annually [2, 7].

1.3 Innovation and regulations in the development of suture threads

Innovation refers to the development of new ideas and improvements, making products, processes, services, technologies, and business models more efficient. Sutures are used for the most basic and complex surgeries, and their usage goes back to ancient times [4, 5, 30]. However, it was until the last 80 years that the majority of improvements have emerged, mostly on the materials, but also with the development of synthetic sutures that are custom-made for a particular anatomic structure and function, which has helped to transform surgery and improve the final outcome, elevating the standards of care [30].

In general, the manufacturing of sutures is under supervision of regulatory agencies such as the Food and Drug Administration (FDA) in the case of the United States of America or the European Medicines Agency (EMA), which classify them as medical devices. However, it is the United States Pharmacopeia (USP), a non-profit, non-governmental agency, the one that provides the manufacturing and testing guidelines for the industry,

for standardization and comparison of suture materials corresponding to metric measures, and also provides guidelines for manufacturing, packaging, sterilizing, and labeling.

The FDA has cleared an increasing number of devices over the past decades, particularly absorbable sutures. Nevertheless, this does not imply that there have been significant improvements in the field, and sutures have barely evolved in terms of technological augment and diversification of function and performance [8, 13].

In this regard, studies like ours [31] raise questions regarding to what extent novel sutures should be evaluated (either preclinically and clinically), as well as to what areas of medical practice could benefit the most from improved suture materials. Abhari *et al*. [8] pointed out the lack of scientific evidence to support the improvements the new sutures claim to offer, concerning effectiveness and safety. These and other authors [32] state that the lack of evidence is due to the regulation under which the new sutures have been approved, for example in the case of the FDA with the 510(k) framework for class II medical devices, for which demonstrating only “substantial equivalence” to an existing predicative device is enough to get the product cleared.

More than 90% of the FDA reviewed devises enter the market by means of the 510(k) pathway, despite since 2011 the Institute of Medicine recommended the replacement of this pathway after concluding that it was inadequate to ensure the safety and effectiveness of medical devises, and inadequate to promote technological innovation as initially conceived. The 510(k) pathway has raised controversy for allowing the commercialization of devises that were subsequently found to carry considerable safety risks [32-34].

The effectiveness of the European and Asian regulatory frameworks for medical devices have been questioned as well. All these three jurisdictions face similar challenges to guarantee the performance and quality of the products, ensuring that only safe and effective devices reach the market [35].

Several reforms have recently been introduced or debated in the United States and Europe that are principally focused on strengthening regulatory processes, enhancing post-market regulation through more robust surveillance systems, and improving the traceability and monitoring of devices [35]. Nevertheless, both jurisdictions approach these challenges in different ways: the United States relies on the FDA, which historically developed as a consumer protection agency, whereas the regulations from the European Commission arose out of a need to harmonize inter-state commercial interests while preserving national "autonomy" [36].

Regulatory framework is very important to take into account considering it directly influences the market by promoting or limiting responsible technological innovation. Practitioners and medical device companies pointed out that certain regulatory process issues might inhibit innovation [32, 37, 38]. Due to the existence of regulations like the 510(k), some authors reported that the *de-novo* process has been rarely used as the regulatory pathway, and medical device companies using this pathway tend to have an unsuccessful experience, mainly due to uncertainty in the process and regarding requirements [38]. Such uncertainty occurs mostly with the newest technologies like the ones needing information technology security (software, depending on network connection, *etc*.) [39], as well as with those using nanotechnology [37], or bioactivated medical devices.

In this regard, no biomaterials targeting cell behavior through biophysical parameters have sought regulatory approval yet to be a test case. Medical devices containing an adjuvant drug substance would be classified as a high-risk class III medical device, adding additional complexity to the regulatory approval process, which can also limit the number of established predicates that can be used to demonstrate equivalence, and with this, the possibility of acquiring the approval via the equivalence-based 510(k) [8]. Although high-risk medical devices have a more complex and costly route to market in both Europe and North America, certain combination devices have gained FDA approval and CE-marked status over the last decade, including: subdermal contraceptive implants, antibiotic bone cement, silver sulfadiazine wound dressings and drug-eluting stents [8].

In an effort to strengthen premarketing testing, the FDA expanded the 510(k) to a "Safety and Performance Based" pathway, which could be potentiated by using "best-in-class" standards when comparing performance and safety, instead of the use of outdated or unsafe predicates, as currently [32].

All stakeholders, which include regulatory agencies, industry, physicians and patients could benefit from improvements in the regulations and communication during the process, since this contributes to reducing the duration of the review process, and would enable to safely bring lifesaving innovations to market faster [38].

The ISO 10993 set involves a series of standards for evaluating the biocompatibility of medical devices. These standards define meticulous aspects such as for sampling and testing *in vitro* cytotoxicity (ISO 10993-5:2009). Nevertheless, the ISO 10993-13:1998 for the identification and quantification of degradation products from polymeric medical

devices does not apply to degradation of the device during its intended use by mechanical stress and wear due to muscle activity, nor biological factors such as enzymes and cellular activity, which are all undisputed when using suture threads.

Our results provide evidence of this to be a necessary ascertainment to carry out. When we cultured cells directly onto commercially available surgical threads, as expected to occur *in vivo* and according to their expected mechanism of action, the studied threads displayed biocompatibility and material properties like degradation significantly different among each other. Such differences can be extrapolated to *in vivo* situations where, they could help explaining common complications. Moreover, an accelerated degradation of the threads would rend them unable to induce significant long-term results when needed.

1.4 Synthetic absorbable barbed sutures

Synthetic sutures are considered inert, and their development allowed to overcome problems observed with catgut, silk and many other materials used before, which displayed inconsistent strength properties and degradation time, thereby triggering a major inflammatory response and causing significant wound irritation and weakness [8, 9].

Theoretically, for most materials, due to their degradation, the tensile strength of absorbable sutures diminishes in tissues about 50% within 60 days. Synthetic absorbable sutures degrade by hydrolysis, when water molecules penetrate into the polymer threads and breakdown the polymer chains of the suture. Furthermore, proteolytic

enzymes easily digest naturally derived absorbable sutures, which are practically completely absorbed within 70 days. In contrast, non-absorbable sutures show poor degradation in body tissues, not being digested by enzymatic action nor hydrolyzed, and hence need to be physically removed [13].

With non-absorbable sutures that persist, a thin fibrous capsule forms, usually by 28 days. With absorbable sutures, if there is an inflammatory reaction it might be more noticeable and continue until the suture is either absorbed or extruded. This is relevant, because the tissue interaction with the braids of the material leads to a down growth of epidermis along the suture path (tissue ingrowth), forming a perisutural cuff. In addition, the non-epidermal tissue around the suture may grow into the suture, and therefore, sutures with the greatest ingrowth and cuff formation will have the most resistance to removal [9].

There is an increasing demand for minimally invasive procedures, and for new sutures, since they play an important role in obtaining a suitable and cosmetic outcome. Recent improvements in synthetic absorbable barbed threads, particularly bidirectional ones, contributed in the development of such less invasive procedures [40].

Bidirectional barbed threads first received clearance from FDA for their use in approximating soft tissues, and they have been available in the market since 2007 [29, 41]. Unlike unidirectional barbed sutures, bidirectional ones do not need a knot at one end to secure the suture; instead, they self-anchor at every 1 mm of tissue through their cuffs or hooks facing the opposite direction of the needle in a helical fashion. This is particularly useful to minimize the amount of material implanted, and with that, the risk of adverse effects. It is also convenient when placing a knot might be difficult, like in deep surgical regions, particularly during minimally invasive surgical procedures [13].

The hooks in bidirectional barbed sutures change direction at the midpoint of the length of the suture, having swaged needles at both ends. They are inserted at the midpoint of a wound and pulled through until resistance from the opposing barbs is encountered, then; each half of the suture is advanced to the lateral ends of the wound creating gradients of evenly distributed tension and compression along the incision line [29, 41-43].

Their design for tissue approximation has the additional advantage of avoiding the need for an assistant's hand to follow the suture placement, as well as a more rapid deployment of the sutures when needed [29].

Due to its decreased effective diameter, the straight-pull tensile strength of barbed sutures is rated one suture size greater than the standard ones. The anchoring of these barbed threads resists migration and is considered as a "continuous interrupted" suture. Therefore, these sutures have been reported to have similar tissue holding performance as compared to knot-anchored sutures, with the possibility of improved cosmesis [29, 42]. Furthermore, they offer a more consistent wound approximation with a reduction in suturing and healing time, economical cost, purse-string effect and other advantages in a variety of surgical settings [29, 30, 40, 41, 44].

Moreover, by eliminating the need to knot, barbed sutures have widened their applications in complex reconstructive surgical procedures. Barbed suture technology has shown to aid soft tissue handling during surgical procedures. In this regard, barbed sutures are efficiently used in various specialties including general and thoracic surgery, obstetrics and gynecology, orthopedics, urology, traumatology, plastic surgery and others [13, 29, 30, 44]. The most common plastic and cosmetic surgery applications include breast surgery, body contouring and hair restoration. Particularly, barbed

sutures have become a popular alternative for minimally invasive percutaneous rejuvenation procedures, such as facelifts techniques [29, 45].

Facelift with barbed sutures involves the passage of threads under the skin of the face and neck to counteract skin laxity and tissue descent. Results of facelift with barbed sutures depend on careful selection of patients with adequate soft-tissue volume, meticulous preoperative planning, application of suitable suturing materials, as well as technical skill and experience. The procedure is expected to produce a biostimulatory effect on collagen formation, inducing some fibrosis and natural tissue stability. Additionally, the procedure is supposed to involve shorter downtime, lower risk of complications and some authors claim it yields aesthetic equivalent results when compared to more invasive facelift procedures [45, 46].

Because of the intervention being less invasive, adverse events do occur, although they are mostly minor, self-limited, and of short duration. Nevertheless, there are no clear data on the long-term effects of the sutures themselves. Moreover, early, as well as long lasting results remain inconclusive.

For other procedures using the same kind of threads, contrary findings have been reported, including relatively higher costs [42], and significantly higher frequency of complications in specific anatomic areas such as the arm and breast [47, 48].

Additionally, barbed sutures differ among each other in specifications such as cut angle, length, depth, and helicity of the barbs, all of which influence their performance, making some sutures more or less suited for treating different tissue types. Nevertheless, it is not considered so when commercializing these products, nor there are wide enough comparisons, contrasting only one or two models at a time [42]

In this regard, some authors claim to feel hesitant because of finding the results and evidence of the use of these sutures to be conflicted [42]. Some other authors have pointed out a lack of objective outcome measures and long-term follow-up data collected in a systematic manner, using for example photographic analysis of facial suspension at fixed intervals postoperatively in a double-blinded fashion, and patients matched for age, sex, and skin characteristics [49]. With our study [31], we found a broad range of results that would correlate with the wide variability reported in the clinical practice even when using devices made of the same material.

For this reason, we agree with other authors who find recommendable to perform studies on the biomechanical and biochemical reactions of barbed sutures in a biological environment and that, until this data is available, surgeons using barbed sutures may wish to apprise patients of the limited data on efficacy, adverse events, and durability of effects [49].

1.5 Bioactive sutures

Although mostly industry driven, the innovation process with regard to suture development, materials and manufacturing techniques along history has broaden the availability of this kind of products. Nevertheless, even when as early as in 1868 there were some attempts of coating sutures with an antibacterial agent (an antiseptic phenol solution) [10], little has been done ever since to enhance the therapeutic value of the sutures themselves. On the other hand, despite the increasing number of sutures passing premarket approval every year, the evidence of the new devices offering clinical

improvements is very limited [8], and wound healing remains a challenging clinical problem [50]. The latter reinforces the interest and the need for innovative effective strategies for both, acute and chronic wound management.

Some of the first significant advances in suture development included the introduction of more developed antimicrobial coatings, the design of barbed sutures and the introduction of new polymer compositions [8, 29]. Most recently, with the rise of regenerative medicine strategies, sutures are slowly shifting from being just inert materials, limited to mechanically approximate tissues, to a bioactive device, which actively promotes cell-directed repair and a better healing response [8, 13]. This is particularly important in complicated wounds, and when there are co-morbidities that may impair a proper healing, *e.g*. in diabetic or infected wounds, or other physiological conditions such as advanced age or inadequate nutrition [17, 18, 51].

Alshomer *et al*. proposed a description for these new bioactive sutures, defining them as "*biomaterials that are engineered to have controlled tissue interaction to optimize wound /defect healing, in addition to their essential function in tissue approximation and ligation*"[2].

The recent advancements and emerging developments in suture materials and technology have found an immense potential in clinical / surgical applications involving specialized procedures and wound management [13]. Besides antimicrobial agents, they allow the addition of bioactive molecules like DNA, antibodies, proteins and growth factors, analgesics and other drugs, or alternatively, the inclusion of cells releasing such molecules [2, 7, 13, 52-59].

Numerous experimental and clinical studies have demonstrated mostly beneficial effects of endogenous and exogenous growth factors, and bioactive molecules such as oxygen, on the final wound healing outcome [60-63].

Growth factors affect a plethora of cellular processes essential for healing, such as proliferation, migration, and differentiation. Consequently, the use of growth factors is a promising approach for achieving optimum tissue regeneration [64-67].

Several growth factors have been proven to be helpful in wound healing processes and in treating chronic wounds, including Vascular Endothelial Growth Factor (VEGF), Platelet-Derived Growth Factor (PDGF) and Stromal Derived Factor 1 (SDF1), also known as C-X-C motif chemokine 12 (CXCL12) [65-80].

VEGF and PDGF genes and polypeptides belong to a family of structurally and functionally related growth factors conserved throughout the animal kingdom [81]. Although their classification into PDGFs or VEGFs is based on receptor binding, it was recently demonstrated that VEGF-A might recognize, bind to and activate PDGF receptors in bone-marrow-derived mesenchymal stem cells [82].

VEGF is considered one of the most potent proangiogenic growth factors, and its concentration in a wound can significantly improve healing [66] or lead to an aberrant outcome [83], therefore, its delivery and stability are key issues to consider. Several Tissue Engineering strategies use rhVEGF or potentiate *in situ* expression of this molecule in order to promote and improve angiogenesis as a crucial component of regeneration [71, 73, 74, 78].

PDGF is involved along the whole process of blood vessel creation, by inducing and supporting angiogenesis, recruiting cells, inducing fibroblasts proliferation and prompting remodeling by means of collagen turnover and cross-linking. Additionally,

PDGF induces vascular smooth muscle cells and pericytes to release angiogenic factors like VEGF, which in turn, synergistically promotes proliferation and migration of endothelial cells [65, 72]. Its clinical efficacy and safety was demonstrated in several phase III studies and, since 1997, the US FDA approved the use of rhPDGF-BB (becaplermin) for the treatment of difficult wounds such as chronic lower extremity diabetic neuropathic ulcers, pressure ulcers, periodontal diseases, and to speed up healing in various surgical procedures [65, 72, 84].

Moreover, SDF-1α is known for its capacity to recruit endogenous progenitor stem cells, particularly hematopoietic ones, but it also enhances the site-specific migration and homing of other cell types like neutrophils, hence improving the healing process and promoting regeneration *in situ* [68, 80, 85]. The binding of SDF-1 to its complementary receptor CXCR4 in combination with other growth factors, has a protective effect of hematopoietic stem cells exposed to γ-irradiation [86].

In spite of all this positive evidence, the short half-lives of the mentioned growth factors and their requirements at specific stages of regeneration make their use in clinical settings challenging [67]. Thus, sophisticated growth factor delivery systems that protect the growth factors from degradation and enable their controlled spatial-temporal delivery are required for their successful translation to viable clinical therapies in regenerative medicine.

On the other hand, oxygen is a crucial molecule with roles as a metabolic nutrient, as well as a signaling molecule [87]. The usage of oxygen in wound healing extends to diagnostic, preventive and therapeutic applications [88].

It is involved in plenty of cellular processes required for tissue homeostasis and regeneration progression such as cell proliferation, collagen synthesis, re-epithelization

and defense against bacteria [60]. In addition to this, ischemia and necrosis predispose the wound to infection, all of which are the principal factors leading to wound dehiscence [29].

Similarly, for engineered tissues, delivering sufficient oxygen to the transplanted cells is one of the most critical issues affecting cell survival and grafting success. Optimizing wound perfusion and ensuring oxygen availability in the perioperative period reduces the incidence of infections, and may elicit some healing responses, as well as synergistically enhance the outcome of other therapies, such as responsiveness to growth factors and acceptance of grafts.

In this regard, while a common goal in tissue engineering is to maximize oxygen supply, studies suggest that moderate oxygenation of cellular scaffolds during *in vitro* conditioning is preferable to high oxygen levels, in a balance that allows to prevent hypoxia while still promoting angiogenesis [89].

Currently, there are several oxygen therapies available, which go from hyperbaric chambers to the use of oxygen generating materials. However, these strategies are expensive and fail to provide oxygen in a consistent manner long enough to allow sufficient time for revascularization [90, 91], for which the oxygen supply should last at least a couple of weeks [87, 90, 92]. Additionally, there are some other concerns about the use of available oxygen releasing materials, mostly about the production of toxic metabolites (*e.g*. hydrogen peroxide, Reactive Oxygen Species, salts, among others) [90].

All this leads to the concept of developing a constant source of oxygen, capable of regulating accordingly to the tissue metabolic requirements. Hereof, by using genetically modified microalgae, we developed the first generation of photosynthetic bioactive

sutures, capable of not only constantly releasing oxygen but also therapeutic recombinant growth factors directly at the wound site, which are expected to enhance the healing process.

2. Methodological strategy

Sutures are widely used to support wounds and tissues within the body. This work aims to contribute to the improvement of surgical threads by means of evaluating commercially available barbed threads, and developing bioactive photosynthetic sutures with improved regenerative capacities. With this in mind, we stress the need of better characterization of suture threads, which could bring higher safety profiles and patient satisfaction. Additionally, we propose that standard commercially available sutures can be bio-activated by the use of microalgae, unicellular microorganisms that we in turn, genetically modified to release both oxygen and selected human growth factors directly to the wound area.

2.1 Analysis of quality variation of some sutures in the market

This section describes the strategy followed to obtain the data presented in the article entitled “PDO threads for facial rejuvenation: analysis of quality variation in the market” [31]. Our study highlights significant variation among six commercially available suture threads in terms of their tensile strength, elasticity, anchoring capacity in human tissue, and biocompatibility.

Suture threads

We evaluated six CE marked threads (21 gauge, barbed) from different manufacturers. The barbs of one of the evaluated threads were press molded onto the filaments, in all other specimen the barbs were cut directly into the material.

Mechanical testing

a. Tensile testing

We evaluated the Young's modulus in a standard tensile test configuration in order to determine the relationship between stress (force per unit area) and strain (proportional deformation) of the evaluated barbed threads. The latter was done by using the uniaxial strain system ZwickiLine Z2.5 (Zwick/Roell, Germany) and a load cell with a maximum loading of 2.5 kN and an accuracy of 0.05% (A.S.T., 10 Germany). During the test, we kept the samples in place using standard compression clamps and inserted for at least 20 mm to prevent slipping out of the clamp during testing. Young's modulus was calculated from the linear range of the stress-strain curve during tensile testing using a speed ramp of 30 mm/min, a sensor recording frequency of 10 Hz, a free length of 30 mm (normalized thread length of 100%) and with regard to the suture diameter.

To determine the maximum load that the threads can hold, we evaluated the absolute maximum values before breakage. Failure (rupture) was evaluated using absolute values in Newton to provide practical results for clinical application.

After mechanical testing, the threads were fixed using 3% glutaraldehyde and kept at RT for further analysis.

These procedures allowed us to notice that, while all threads evaluated are sold as 21 gauge, the products span a considerable range of diameters, leading to significant

differences in the maximum tensile strength across the barbed threads, reinforcing the assumption that the different core diameters (0.3-0.4 mm) significantly affect the absolute strength of the threads. Regarding to the Young´s modulus, we found less pronounced differences among threads, coinciding with the assumption that the basic material composition of all products is comparable.

b. Ex-vivo pull-out test

As a complementary mechanical evaluation, we performed an *ex-vivo* pull-out test. For this, the threads were injected into human skin samples to evaluate their anchorage behavior/lifting abilities during the pull-out testing. The pull-out strength was represented as the construct stiffness before failure as well as the maximum pull-out force declared as the failure point. All experiments were performed in accordance with relevant guidelines and regulations. To use the human skin, we obtained the approval of the Ethics-Committee of the Klinikum rechts der Isar of the Technical University of Munich, Germany (Application number: 204/17 S), and obtained informed consent of donors. To avoid individual differences, abdominal skin samples were collected from one donor. After washing and disinfecting the tissue samples, subcutaneous fat tissue was removed carefully. The remaining skin and subcutaneous fibrous tissue was cut into five smaller pieces of 3x6 cm^2 each and kept hydrated with PBS at all times. Each of the five skin samples was injected with all six evaluated thread types. Threads were injected by a trained plastic surgeon in the same way as in clinical practice and according to the manufacturer's instruction. A gap of 10 mm for the skin/thread implantation site remained free of clamping in order to avoid an additional compression of the specimen (skin and implanted barbed thread). The first force drop after the linear range was

defined as anchorage failure and later this value was labeled as the maximum force exercisable by a specific thread. After mechanical testing, the threads were fixed using 3% glutaraldehyde and kept at RT for further analysis.

This assay was required to study the deformation of the barbs in the threads, and allowed us to identify the threads with smaller anchoring/lifting capacity. In this way, threads with small and strong barbs distributed closely to each other showed the best anchorage in the human tissue.

Biocompatibility testing

The ISO 10993 set is a result of an international harmonization process that describes standards for evaluating the biocompatibility of medical devises aiming to guarantee their safe use. More specifically, ISO 10993-5:2009 specifies test methods to assess the *in vitro* cytotoxicity of medical devices, and ISO 10993-6:2016 describes test methods for the assessment of the local effects after implantation of biomaterials intended for use in medical devices.

In an attempt to bring closer to each other this two sets of international standards and to the daily practice, we performed an assay culturing human cells directly onto the evaluated threads, using $10 \cdot 10^5$ HS-27 human fibroblasts and 1cm thread pieces per well in 24-well plates. Culture medium was changed once a week. After four weeks in culture, a live/dead staining was performed using the"ReadyProbes® Cell Viability Imaging Kit (Ref. R37610) according to manufacturer's instructions. Furthermore, metabolic activity of cells growing on the threads was measured by reduction of MTT. Therefore, each thread piece was placed in 250μL of culture media containing 10% of MTT reagent (Sigma, M 5655) and incubated for 3 hours at 37°C. Supernatant was

replaced with 250µL of DMSO and optical density was measured at 560 nm. DMSO was used as blank and unseeded thread pieces as negative control.

Additionally, toxicity of the material was evaluated by measuring lactate dehydrogenase (LDH) release using the same cell line. Again, culture medium was changed once a week and medium samples were collected between weeks three and four and centrifuged 5 min at 300g to perform the assay (Roche, Cat. 11644793001). Culture medium was used as blank and supernatant of cells not exposed to the threads was used as a negative control. The remaining seeded threads were fixed for SEM.

The methodology described here allowed us to identify significant differences among the evaluated sutures, in terms of viability, metabolic activity as well as in LDH release.

Scanning Electron Microscopy (SEM)

Threads directly of the shelf and from tensile, pull-out, and biocompatibility testing were dehydrated with graded ethanol and air-dried at room temperature (RT). The dehydrated threads were then sputtered with Gold (BAL-TEC SCD 005) 40 sec at 40 mA, 8 nm, and imaged, using 10 kV (JOEL JSM 6390).

The use of this technique allowed us to obtain further evidence, complementary to the mechanical and biocompatibility testing.

Statistical analysis

The evaluation of the data was performed by using One-way analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons. $p<0.05$ was considered as statistically significant. Statistical evaluations and bar graphs were generated using GraphPad Prism Version 6 (GraphPad Software, La Jolla, CA/USA).

This methodological strategy allowed us to identify significant differences in material performance and biocompatibility of six CE marked barbed PDO thread products. The insufficient performance of some products may be linked to severe side effects observed in clinical outcomes. Therefore, the results of this study are relevant for clinicians interested in minimal invasive procedures like facial rejuvenation. The material and safety data presented may enable improved thread design and inform clinical decision making.

2.2 Bioactive surgical threads

We propose that standard commercially available surgical threads can be bio-activated with genetically modified microalgae to release both, oxygen and recombinant growth factors directly into the wound area. We find this to be valuable considering wound healing is characterized by diminished levels of blood perfusion and that, among the blood components, oxygen and proregenerative growth factors are broadly described as key players for the healing process.

The present section resumes the strategy followed to obtain the data presented in the paper entitled “Development of photosynthetic sutures for the local delivery of oxygen and recombinant growth factors in wounds” [93]. Briefly, it covers the process to genetically modify the microalgae, as well as its culture, distribution, and proliferation capacity when seeded onto commercially available sutures. Additionally, we evaluated the mechanical properties of seeded sutures compared to unseeded controls, as well as

the oxygen production and recombinant growth factor release over time. Finally, photosynthetic sutures were tested in order to evaluate their resistance to mechanical stress and freezing. Our results suggest that photosynthetic gene therapy could be used to produce a new generation of bioactive sutures with improved healing capacities.

Cell culture of the microalgae (C. reinhardtii) and seeding in the threads

C. reinhardtii was chosen due to its handling and safety advantageous characteristics [94]. Cell-wall deficient strains UVM4-GFP and UVM4- VEGF [95] were grown photomixotrophically on either semisolid Tris Acetate Phosphate (TAP) medium or in liquid TAP medium supplemented with 1% (w/v) sorbitol and kept under continuous white light exposure (2000 lx, ≈30 mE), using a lamp with the full spectrum of light.

Polyglactin 910 undyed, braided, gauge size 0, 3–0 and 5–0 Vicryl suture threads (Ethicon, NJ, USA) were used (3.5, 2, and 1 according to European Pharmacopoeia, equivalent to 0.35, 0.2 and 0.1 mm respectively). The strings were cut into 3 cm pieces under sterile conditions, winded and submerged into a $5 \cdot 10^7$ cells/mL microalgae solution and incubated at room temperature under constant illumination (2000 lx, ≈30 mE) with no agitation. After 24 h, *C. reinhardtii* seeded thread pieces were transferred to a new petri dish with fresh TAPS medium and kept in culture for 1, 4, 7, 10 and 14 days adding fresh TAPS every 3 days.

Light and confocal imaging pictures of the threads at different time points were taken, and then analyzed with the AxioVision SE64 Rel. 4.9.1 software (Carl Zeiss AG, Oberkochen, Germany).

All sutures showed an overall green color, which was homogeneously distributed along the entire material, and showing an increase in the intensity of the green color over time.

Chlorophyll measurement

As described, we seeded pieces of each evaluated gauge threads with *C. reinhardtii* cells and incubated them for 1, 4, 7, 10 and 14 days at room temperature under continuous light exposition. Then, sutures were placed in 250 µL of dimethyl sulfoxide (DMSO) and put under agitation for 10 min. After that, the optical density was measured at 435 nm (Nanodrop, Thermo scientific, Waltham, MA, USA). Unseeded threads kept under the same conditions were used as blank.

The increase in the intensity of the green color observed over time concurred with increasing amounts of chlorophyll content at each time point for up to 10 days in culture. Furthermore, the results showed a direct correlation between the suture size and its chlorophyll content.

Based on the results obtained for the chlorophyll content, all further experiments were performed using 0.35 mm threads in order to maximize the number of algae within a photosynthetic suture.

Scanning electron microscopy (SEM)

Suture threads were seeded with the microalgae and incubated for 7 days as described before. After fixation in 3% glutaraldehyde and dehydration with graded ethanol, samples were air-dried and sputtered with gold to 20 nm thickness (Baltec, SCD 005;

Leica Microsystems, Wetzlar, Germany). A voltage of 3.0 kV was used for the scanning electron microscopy analysis (JSM 6390, Jeol, Tokyo, Japan).

This analysis confirmed the presence of algae in all filaments, in most cases forming microcolonies.

Oxygen release quantification

Oxygen concentration *in vitro* was measured by placing the thread pieces in Oxodishes®, and using the Sensor Dish® Reader (SDR; PreSens GmbH, Regensburg, Germany) according to the manufacturer's instructions. Twelve centimeter thread pieces were seeded with *C. reinhardtii* as described above, kept in culture for 1, 4, 7, 10 days and placed in the Oxodishes. The Sensor Dish with the Oxodish and samples were kept inside an incubator at 1% oxygen, 35 °C and 100% humidity during the length of the experiment. For light stimulation, a lamp with the full spectrum of white light was placed 40 cm away from the sample. Oxygen concentration was recorded, and measurements stopped when saturation of the system was reached and maintained for at least 1 h. The results show the amount of time (hours) that it takes for each condition to reach and maintain the maximum oxygen concentration that is possible to measure with the PreSens-system. This maximum amount of oxygen corresponds to 52.3% pO2, which is equivalent to 250% of the amount of oxygen present in air saturated water (250% air sat.). This assay correlated with the observation of bubbles formation from the seeded threads.

Co-culture with fibroblasts/HIF-1α quantification

Seeded sutured pieces were kept in culture for 10–14 days and then coated with a fibrin glue solution to prevent microalgae growth outside the thread segment. 3T3 fibroblasts ($1 \cdot 10^6$ cells per well) were seeded in six-well plates and after 24 h, 36 cm photosynthetic coated threads were placed in each well and kept under light exposition and hypoxic conditions (1% oxygen) at 35 °C for 16 h with 2 mL culture media (1:2 TAPS and DMEM without phenol red, with glutamine and 10% FCS). Control consisted of 3T3 cells kept under same conditions but using unseeded threads. Afterwards, supernatant was removed quickly and placed in liquid nitrogen. Cell extracts were obtained by lysing the cells with 200 µL lysis buffer specified by the manufacturer in the ELISA kit. Total protein extracts were quantified by BCA assay. Then, Hypoxia inducible factor 1-alpha (HIF-1α) was quantified by ELISA (DYC1935-2, R&D Systems, Minneapolis, MN, USA). With this assay we found that, when co-cultured with 3T3 fibroblasts under light exposition and hypoxic conditions at 35 °C, oxygen released by the microalgae in the seeded threads induced a significant reduction in the levels of the hypoxic marker HIF-1α expressed by the animal cells.

Gene modification of microalgae

As described previously by our group [95], the coding sequences for the human stromal cell-derived factor 1 (accession number P48061) and human full-platelet-derived growth factor subunit B (accession number P01127) were adapted to the codon bias of *C. reinhardtii* and inserted into the transformation vector.

The final constructs were verified by sequencing. The plasmid DNA was replicated and purified from transformed *E. coli* K12 (dam + dcm + tonA rec–) bacteria, and the plasmid

concentration determined by UV spectroscopy. After transformation, cells were seeded in TAPS liquid medium and incubated overnight in the dark with continuous shaking. Then, the algae were seeded on TAP-agar plates containing 10 mg/mL paromomycin and incubated for the first three days under dim light. The plates were then moved to standard light exposition (2000 lx, ≈30 mE) until the paromomycin resistant colonies were large enough to be transferred to a fresh plate. Clones were subsequently maintained on solid TAP medium under selective conditions.

The integration of the recombinant gene was confirmed by PCR using gene specific primers pairs (Metabion GmbH, Planegg, Germany; and Sigma-Aldrich, Taufkirchen Germany).

In a complimentary effort, we implemented and compared three different promising approaches in the search for the optimal combination of algal-strain and expression vector that would lead to the best secretion yields of our *C. reinhardtii*-expressed recombinant human growth factors [96].

Cytokine release from photosynthetic sutures

Genetically modified algae were used to seed thread pieces and kept in culture for 1, 4, 7, 10, and 14 days. At each time-point, threads (9 cm) were transferred to new wells with fresh TAPs liquid medium, and 24 h later, supernatants were collected, centrifuged and stored in -80 °C. The concentration of the recombinant growth factors in the supernatants was measured using the human VEGF, PDGF and SDF-1a Enzyme-linked Immune Sorbent Assays (ELISAs) respectively (R&D Systems, Minneapolis, MN, USA: VEGF Quantikine® ELISA kit (DVE00), Human PDGF-BB Quantikine® ELISA kit (DBB00), and Human CXCL12/SDF-1 DuoSet ELISA kit (DY350)).

<u>In vitro</u> activity of released cytokines

$1 \cdot 10^5$ Human Umbilical Vein Endothelial cells (HUVECs, PromoCell GmbH, Heidelberg, Germany) were seeded per well on a 12 well-plate, cultured for 24 h and then starved for 16 h before activation. Similarly, human Adipose-derived Stem Cells (hu ASC) (PT-5006, Lonza, Basel, Switzerland) were seeded in 12 well plates ($6.5 \cdot 10^4$ per well) or in 96 well-plates ($0.3 \cdot 10^4$ per well), cultured for 24 h and starved for 16 h using RPMI 1640, with stable glutamine and 2.0 g/L NaHCO3 (Biochrom, Berlin, Germany) supplemented with 1% fetal calf serum (heat inactivated FCS, Biochrom GmbH, Berlin, Germany) and 1% antibiotic/antimycotic (100x ab/am; Capricorn Scientific, Ebsdorfergrund, Germany). Recombinant proteins were recovered by passing 30 mL of the supernatants from confluent *C. reinhardtii* cultures (ca. $2 \cdot 10^7$ cells/mL) through a 0.22 µm filter, followed by a centrifugation step (3000g, 47 min) using filtration tubes capable of retaining peptides above 30 kDa (Vivaspin 15R Hydrosart, Sarstedt, Goettingen, Germany). In another filtration step under the same conditions, the diluent medium was changed to RPMI 1640. Afterwards, the media were supplemented with 1% fetal calf serum (heat inactivated FCS, Biochrom GmbH, Berlin, Germany).

In order to evaluate the biological activity of the released cytokines (rhVEGF, PDGF and SDF-1α), a receptor phosphorylation assay was performed. For this purpose, the starved cells were stimulated for 5 min, with either 50 ng/mL recombinant VEGF-165 (Preprotech, NJ, USA), or the concentrated protein supernatants of confluent cultures of the genetically modified or wild-type strain. Cells were then snap-frozen by submerging the plate in liquid nitrogen and lysed in RIPA-buffer with phosphatase inhibitors and proteinase inhibitors. Cells were scratched from the well-floor, and lysates were homogenized by pipetting up and down and stored at -80 °C for further analysis.

Detection of receptor and phospho-receptor was performed in a semi-quantitative way by using a Human Phospho-VEGF R2/KDR DuoSet IC ELISA kit (cat. DYC1766-2, R&D Systems, Minneapolis, MN,

USA). For the evaluation of PDGF, the same procedure was performed but using huASCs, 10 ng/ml recombinant PDGF-BB (Preprotech, NJ, USA), and Human Phospho-PDGF R beta DuoSet IC ELISA kit (cat. DYC1767-2, R&D Systems, Minneapolis, MN, USA). For SDF-1a, huASCs, 30 ng/mL SDF-1a/CXCL12 (Preprotech, NJ, USA) and CXCR4 Colorimetric Cell-Based ELISA Kit (CBP1352, CytoGlowTM, Sunnyvale, CA, USA) were used according to instructions from the manufacturer.

When performing this methodology we found the genetically modified microalgae were capable of releasing recombinant growth factors for up to 14 days *in vitro*. Additionally, the supernatants of the seeded threads stimulated the cells in culture, and the bioactivity of the released growth factors was confirmed by the phosphorylation level of its respective receptor in HUVEC (rhVEGF) or mesenchymal stem cells (rhPDGF and rhSDF-1).

Biomechanical characterization

Seeded threads measuring 7–8 cm were kept in culture for 1, 7 and 14 days as previously described. Non-seeded sutures were incubated under the same conditions as controls. Sutures were subjected to a tensile strength test using a uniaxial digital Electronic Dynamometer D500 (Industria HP, Argentina). Sutures were clamped at both ends and the total length of the suture was recorded. Then, the sutures were loaded at a constant rate of 30 mm/min until failure, and the applied force and displacement were recorded throughout the whole process. Elongation was calculated by correcting displacement by

the total length of each sample, which was then used in conjunction with the applied force to calculate the maximum force and elasticity. Samples were tested one after the other at a controlled temperature of 20 °C, and were kept hydrated at all times.

Taking into consideration that the therapeutic potential of surgical sutures relies on their mechanical properties, we consider these assays essential. Our results showed that the suturing material retains most of its original properties at different times after seeding; hence, showing that neither the seeding process itself nor the presence of the algae compromise their main function as tissue holders.

***Ex vivo* evaluation of photosynthetic threads**

C. reinhardtii were seeded as described before in commercially available polyglactin 910 undyed, braided, gauge size 0 Ethicon Vicryl suture threads (70 cm long), and kept in culture for 14 days. Threads were then used to evaluate their integrity, algae content and algae survival, after being used to suture human skin *ex vivo*. For this, photosynthetic threads were passed through full thickness human skin to resemble 45 stitches. Before the first stitch and after each following stitch, 1 cm of the thread was cut and placed in 250 µL DMSO to quantify its chlorophyll content. Additionally, before the first and after the last stitch, another 1 cm piece was cut and placed in TAPS agar plates containing paromomycin to determine algae viability.

We found that most of the microalgae remained inside the threads and that the chlorophyll content was not significantly diminished after performing up to 45 stitches. The skin used for these experiments was abdominal full thickness human skin, harvested during abdominoplasty procedures. The skin was transferred directly from the operating

room to the laboratory to perform the experiments. The local ethical committee approved the study (204/17S), and informed consent was obtained from the donors.

Cryopreservation

In order to evaluate the feasibility of cryopreserving photosynthetic sutures, seeded fragments (3 cm length) were incubated for 10 days as described before. Four fragments were placed individually in 0.5 mL of erythrosine solution (0.04 $g \cdot L^{-1}$) for 10 min at room temperature and then centrifuged (5 min, 14,100g). Four other fragments were placed in cryotubes containing TAP medium + 10% methanol as freezing medium for the algae, and then transferred to a Nalgene container and incubated at -80 °C for 24 h. Afterwards, sutures were thawed and transferred to new tubes containing 0.5 mL of erythrosine solution for 10 min at room temperature and centrifuged. Another four pieces were placed in cryotubes with TAP medium + 5% Sekusept, and incubated at 37 °C for 24 h as a control for death algae.

One fragment from each group was placed in a TAPs agar plate and kept at room temperature under continuous light stimulation for 2 weeks. Viability of the algae in the threads was determined by a colorimetric assay, which relies on the selective adsorption or uptake of erythrosine by non-viable cells, hence, supernatants of the erythrosine solution were measured at 526 nm. Unseeded threads were used as a blank control, showing no adsorption of the reagent and the highest absorbance values.

We found no significant differences in erythrosine adsorbance of photosynthetic sutures that were kept at room temperature or frozen. However, we did find statistical differences among the previous groups compared to the positive dead control, in which seeded cells were previously stressed to death. Additionally, the capability of algae to

grow from the frozen photosynthetic sutures into agar plates confirmed their viable status.

Statistical analysis

All assays were performed in at least three independent experiments. The data were expressed as mean ± SD. The GraphPad Prism 7 software (GraphPad Software, La Jolla, CA, USA) was used for statistical analysis. One-way ANOVA, or Two-tailed Student's t-tests were performed to evaluate the differences among groups. Differences among means were considered significant at $p \leq 0.05$.

Although further experiments are required to evaluate the safety and efficacy of this technology *in vivo*, this work represents the first step to create a new generation of surgical sutures with improved regenerative capabilities.

3. Discussion

As mentioned above, not only the physician's expertise and the patient's physiology are important for a good healing outcome, but also, the suture thread itself, along with its intrinsic physical and mechanical characteristics may influence the ultimate result.

With this work, we intended to highlight the need of a better characterization of suture threads in terms of even their most common characteristics. In this regard, despite their approved license to be commercialized, and even when they are made of the same material, the threads available in the market show significant differences regarding their microstructure, tensile strength, elasticity, anchoring capacity in human tissue, and biocompatibility. For studying this, we focused on barbed threads frequently used for minimally invasive cosmetic procedures.

Moreover, we proposed a photosynthetic therapy strategy to bioactivate absorbable suture threads, and evaluated its feasibility *in vitro*. The strategy looked for enhancing the healing process in incisional wounds, through the incorporation of genetically modified microalgae capable of releasing oxygen and human growth factors.

The results of both studies are highly relevant for clinicians and patients. They elucidate some of the factors that might be linked to the occurrence of various typical complications of minimally invasive procedures using barbed threads (*e.g*. facial rejuvenation treatments), such as superficial displacement of the sutures, dehiscence, seroma, erythema, necrosis, infection, skin dimpling, and others. In the case of the photosynthetic gene therapy approach, our results open an opportunity to successfully

treat incisional wounds, particularly in those patients who tend to have a more hypoxic tissue environment and difficulty for healing.

3.1 PDO threads for facial rejuvenation

Facelift procedures using barbed suture insertion have gained much attention as a minimally invasive option. The procedure is expected to provide the same results as other more invasive alternatives, with advantages like reductions in costs, in recovery time, risk of complications, among others [45]. Nevertheless, a great diversity of clinical outcomes was found in several case series, reports and small clinical trials [97-101], and in general, results of barbed suture facelift seem not to be consistently favorable and long-lasting.

With the idea of creating awareness about the need of robust scientific evidence about the effectiveness and safety of the newer materials and designs, our group prepared the scientific publication entitled “PDO threads for facial rejuvenation: analysis of quality variation in the market” [31]. In this study, we suggest that the reason for the variability of the results and the occasional complications relayed on differences in the suturing material used, which evidences the need for a comprehensive testing and analysis of the barbed synthetic absorbable sutures in terms of their production and mechanical properties, biocompatibility and biodegradability, which definitely contribute to their performance.

Most commonly, barbed threads for facial rejuvenation are made out of PDO, a polymer made from the poly(paradioxanone)polyester, manufactured as monofilamentous

sutures and eliciting a minimal foreign-body reaction [9, 45]. In this regard, our group performed an analysis of quality variation of six different PDO threads for facial rejuvenation available in the market. In this study, we analyzed their microstructure, tensile strength, elasticity, anchoring capacity in human tissue, and biocompatibility, finding significant variations among the threads in each of the studied aspects. It would be a logical assumption that all CE marked barbed PDO threads have similar characteristics and attributes, but according to our results, this is not the case. Despite their license to be marketed and sold in the European Union, and the fact that all of them are supposed to be composed of the same kind of polymer, some products performed significantly worse than others on material testing, and even displayed cytotoxic characteristics, as detected with a lower metabolic activity, and higher release of lactate dehydrogenase by a human fibroblasts cell line when cultured on the threads *in vitro*.

On the other hand, while four out of the six evaluated products showed to be not or only slightly affected when kept in the culture medium of the of fibroblasts, two of them were completely dissolved after being in culture for four weeks [31]. The retention profile of PDO in the literature, as expressed by approximate percentages of the initial retention strength is of 70% by day 14, 50% at day 28, and 25% after 42 days [10]. For producing the cosmetic effect, the threads are expected to display enough strength to hold the tissues against gravity and muscle movements, as well as to last long enough until new collagen has been synthesized in the surroundings, allowing the self-stability of the tissues [45]. Nevertheless, if the threads are dissolving in a considerably shorter time, it directly affects the possibility of providing enough strength and time for

fibroblasts to expand and produce the needed collagen and other extra cellular components.

Furthermore, we studied how barb morphology influences the grasping of the threads to the tissues and their tensile strength. We found out that the deeper the barb is cut, the lower the tensile strength [29]. In addition, within the six studied threads, the ones with smaller and stronger barbs distributed close to each other showed the best anchorage in the human tissue. Finally, we noted that, even though the evaluated threads had different diameters, they all used the same needle size, which as a result had a direct effect in the suture anchorage potential, as shown in the "pull-out *ex vivo* assay". Here, we concluded that, using smaller diameter needles results in better adherence of the soft tissue to the barbs, because a wider channel is not produced by a larger diameter needle [29].

Contribution of the author

The author contributed by designing the assays, performing the experimental work to study the biocompatibility of the sutures. Furthermore, the researcher Centeno-Cerdas collaborated on the microscopy images acquisition, the biomechanical testing of the sutures, and was involved in the overall analysis of the results and manuscript writing.

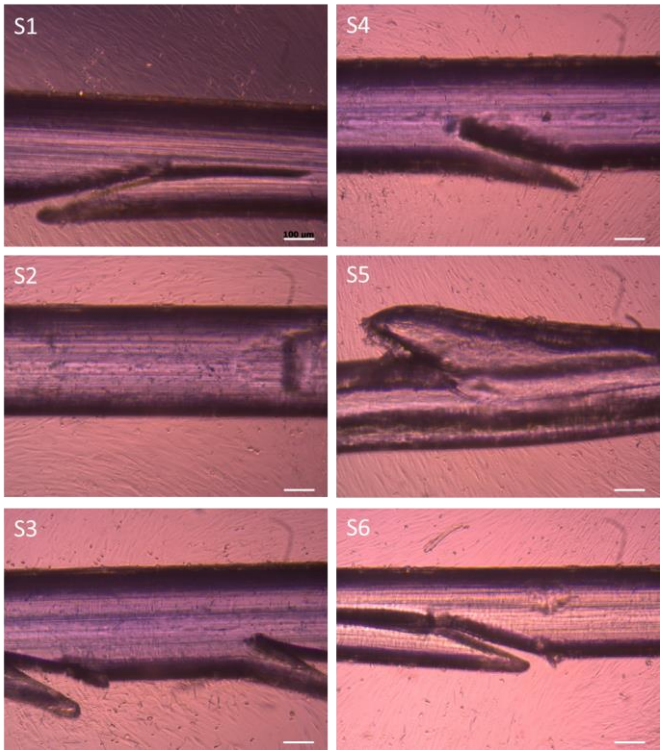

Figure 1. Six different commercially available suture threads were placed in culture with HS-27 human fibroblast cell line in order to evaluate the metabolic activity and cell stress of the fibroblasts when growing on the thread material. The pictures were taken after two weeks in culture, with a Zeiss microscope and analyzed with the AxioVision SE64 Rel. 4.9.1 software (Carl Zeiss AG, Oberkochen, Germany). Scale bars represent 100 µm.

In summary, while barbed threads have become a useful medical device in multiple minimally invasive, wound healing and cosmetic approaches [13, 29, 45], there is the need of more exhaustive testing of their mechanical properties, biocompatibility, as well as standardize comprehensive evidence of their long lasting effects.

3.2Photosynthetic materials as adjuvants for wound healing

Bioactive sutures explode the advantage of a local release, directly at the wound site either by coating the surgical threads with active molecules or by seeding cells releasing them [2, 52, 53, 56, 57]. Some of these coated sutures approaches have shown success, yet there are many in which, the inclusion of the molecules or cells affected the tensile strength of the suture [2, 102]. On the other hand, in others, the mechanical stress of the sewing process led to physical disruption of the bioactive reagent [54, 56], and therefore, limited the rate and yield of drug release.

The concentration of some key molecules is a determinant aspect leading to a diminished, improved or aberrant outcome. Then again, many bioactive molecules display a short life, limiting their use. Therefore, there is a major need to find a proper strategy to introduce lasting biomolecules carriers into the sutures, or alternatively, delivery methods capable of tuning according to the tissue metabolic requirements.

Tissue defects characterize by the disruption of the vascular network, which causes a scarce of oxygen supply, and leads to scarring rather than regeneration, due to the oxygen demand for cell proliferation, collagen synthesis, re-epithelization and defense against bacteria [60, 87]. Additionally, ischemia and necrosis predispose the wound to infection, which are the important factors leading to wound dehiscence [29].

Recently, our group introduced an approach named HULK (from the German *Hyperoxie Unter Licht Konditionierung*). HULK has proven to be effective in delivering oxygen independently of blood vessel perfusion, as well as recombinant growth factors to aid the wound healing process by means of incorporating photosynthetic microalgae such as *Chlamydomonas reinhardtii* (*C. reinhardtii*) [103, 104]. This alga is a well-characterized

model organism, categorized by the U.S. FDA as “Generally Regarding as Safe” (GRAS), and previously shown not to elicit a significant immune response when implanted in a murine host. Moreover, *C. reinhardtii* is well known for its ease of cultivation, and suitability for the production of recombinant complex proteins [95, 103-106].

Considering this, we seeded photosynthetic cells into surgical threads and proposed to use them as a local and permanent source of oxygen and growth factors during wound healing. Specifically, we introduced the bioactivation of collagen matrixes and polyglactin 910 surgical threads with genetically modified microalgae, in order to deliver both, oxygen and other pro-regenerative molecules directly into external wound sites [95, 106, 107] or incisional cuts needing mechanical fixation [93].

Based on promising evidence, we selected three growth factors: VEGF, PDGF, and SDF-1. These molecules have been proven to be helpful in wound healing processes by supporting angiogenesis and endogenous progenitor stem cells recruitment [68, 72, 108].

The results of this project were published under the title “Development of photosynthetic sutures for the local delivery of oxygen and recombinant growth factors in wounds” by Centeno-Cerdas *et al* [93] and “Towards autotrophic tissue engineering: Photosynthetic gene therapy for regeneration” by Chavez *et al* [95]. The retention period of the used polyglactin 910 surgical threads is 28 to 35 days, which expressed as percentage of initial retention strength corresponds to 75% after 14 days, and 50% after 21 days, with an absorption rate of 56 to 70 days [10]. Our results show that, neither the algae nor the seeding protocol significantly modify the elastic limit, maximum force and the Young´s module of the suture. The only exception was observed for the maximum force at day 14, where a significant decrease was observed for the photosynthetic

material [93]. We further demonstrated that the use of genetic engineering tools allows photosynthetic biomaterials to deliver additionally recombinant growth factors *in situ* [95].

We concluded that this approach is a promising first step towards engineering photosynthetic surgical materials with pro-regenerative features, which will contribute to the establishment of new therapeutic approaches for the treatment of skin wounds.

Contribution of the author

The doctoral candidate contributed to the planning, performance, analysis and writing processes that led to the publication of the scientific paper of Centeno-Cerdas *et al*. (2018) [93]. More specifically, designed and executed chlorophyll measurements and viability assays in order to define the best method to inoculate the suture threads with the microalgae *C. reinhardtii*. Here, several seeding strategies and inoculum were evaluated, including injecting the microalgae directly and horizontally along the core of the sutures, incubating overnight under orbital agitation in highly concentrated algae solutions, and widing and dipping the threads in algae liquid cultures, which turned out to be the most efficient method (Figure 2). Furthermore, the author performed as well the chlorophyll measurements and oxygen release quantification to follow the algae population behavior over time when growing on and within the threads. She obtained the light, confocal and scanning electron microscopy images, and also executed the statistical analysis, performed the comprehensive analysis of the results and the drafting of the manuscript.

3.3 Other published contributions

The author also contributed in the experimental design and data acquisition of close related published works [95, 96] and other regenerative medicine strategies (presented as oral talks and posters in the editions of 2017 and 2018 of the Deutschen Gesellschaft der Plastischen, Rekonstruktiven und Ästhetischen Chirurgen (DGPRÄC), and the Deutschen Ästhetisch-Plastischen Chirurgen (VDÄPC).

The author participated in the preclinical evaluation of other medical devices and photosynthetic gene therapy adjuvants for dermal tissue regeneration, as described in the article “Towards autotrophic tissue engineering: Photosynthetic gene therapy for regeneration” by Chávez *et al* [95]. Additionally, in the work by Jarquín-Cordero *et al*, we implemented and compared three different promising approaches in the search for the optimal combination of algal-strain and expression vector that would lead to the best secretion yields of our *C. reinhardtii*-expressed recombinant human growth factors [96].

Both efforts included the quantification of recombinant growth factors and the validation of their bioactivity through cell proliferation and migration assays.

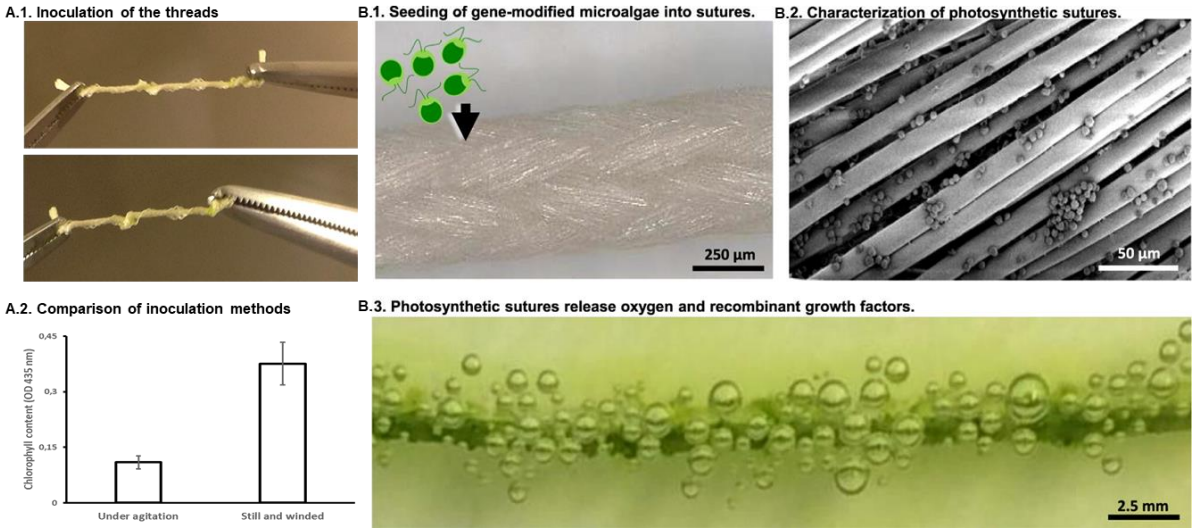

Figure 2. Strategy followed in the development of the photosynthetic sutures. Comparison of two of the seeding methods for incorporation of microalgae *C. reinhardtii* onto polyglactin 910 surgical threads. Chlorophyll content was measured 24 hours after inoculating the threads either under agitation or by winding them. The results show a significantly higher amount of chlorophyll using the second method, thereby reflecting a higher number of algae retained inside the suture (**A 1 and 2**). Once the method for seeding was chosen, genetically modified algae were added, (**B1**), and the photosynthetic sutures were characterized in terms of their biomechanical properties (**B2**) and bioactivity of the molecules released (oxygen and growth factors) (**B3**). Modified from the graphical abstract of Centeno-Cerdas *et al*., available in https://www.sciencedirect.com/science/article/pii/S1742706118305890?via%3Dihub

4. Closing remarks

Thread sutures are important medical devices for wound management that in recent years have expanded their applicability and efficacy.

The availability of a growing amount of products can make the proper choice for closure a challenge, and in order to make a better, more informed decision, a more comprehensive characterization of suture materials is needed, even when been produced with the same biomaterial.

Moreover, there is an immense potential for next generation of suture materials, as an outcome of multidisciplinary efforts, to positively influence surgical outcomes and wound management.

In this regard, the possibility to use as carriers to deliver drugs, stem cells, proteins, growth factors, antibodies, nucleic acids, nanoparticles, among others, into a desired anatomical location is drastically enhancing the therapeutic potential of suture threads.

The results of our studies are relevant for biological engineers, clinicians and patients. Our findings suggest that photosynthetic gene therapy could be used to produce a new generation of bioactive sutures with improved healing capacities. This information, along with the material and safety data presented for the materials already in the market, may enable improved thread design and informed clinical decision making.

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6. Doctoral candidate's list of publications

Following peer-review processes, the research performed during the doctoral studies and described in this document was accepted to be divulged in renowned scientific journals and international events as follows:

M. Jarquín-Cordero, M.N. Chávez, **C. Centeno-Cerdas**, A.-V. Bohne, U. Hopfner, H.-G. Machens, J.T. Egaña, J. Nickelsen, Towards a biotechnological platform for the production of human pro-angiogenic growth factors in the green alga Chlamydomonas reinhardtii, Applied Microbiology and Biotechnology 104(2) (2020) 725-739.

C. Centeno-Cerdas, M. Jarquin-Cordero, M.N. Chavez, U. Hopfner, C. Holmes, D. Schmauss, H.G. Machens, J. Nickelsen, J.T. Egana, Development of photosynthetic sutures for the local delivery of oxygen and recombinant growth factors in wounds, Acta Biomater 81 (2018) 184-194. (DOI: doi.org/10.1016/j.actbio.2018.09.060)

Matthias M. Aitzetmueller*, **Carolina Centeno-Cerdas***, Phillipp Nessbach, Peter Foehr, Elizabeth Brett, Dominik Thor, Hans-Guenther Machens, Rainer Burgkart, Dominik Duscher. Polydioxanone Threads for Facial Rejuvenation: Analysis of Quality Variation in the Market, Plastic and Reconstructive Surgery 144(6) (2019) 1002e-1009e.

(*sharing first co-authorship).

M.N. Chavez, T.L. Schenck, U. Hopfner, **C. Centeno-Cerdas**, I. Somlai-Schweiger, C. Schwarz, H.G. Machens, M. Heikenwalder, M.R. Bono, M.L. Allende, J. Nickelsen, J.T. Egana, Towards autotrophic tissue engineering: Photosynthetic gene therapy for regeneration, Biomaterials 75 (2016) 25-36. (DOI: dx.doi.org/10.1016/j.biomaterials.2015.10.014)

Carolina Centeno-Cerdas, Montserrat Jarquín-Cordero, Myra Noemi Chávez, Ursula Hopfner, Daniel Schmauß, Jörg Nickelsen, Hans-Günther Machens, José-Tomás Egaña. "Photosynthetic sutures with pro-regenerative features". Poster presented in the Tissue Engineering and Regenerative Medicine International Society (TERMIS) congress, European chapter, from 26th to –30th of June in Davos, Switzerland.

Carolina Centeno-Cerdas, Montserrat Jarquin**,** Myra N. Chavez**,** Ursula Hopfner**,** Daniel Schmauss**,** Jörg Nickelsen**,** Hans-G. Machens**,** Tomas J. Egana. "Photosynthesis induced pro-regenerative sutures". Oral talk presented in the 48. Jahrestagung der Deutschen Gesellschaft der Plastischen, Rekonstruktiven und Ästhetischen Chirurgen (DGPRÄC), 55. Jahrestagung der Österreichischen Gesellschaft für Plastische, Ästhetische und Rekonstruktive Chirurgie (ÖGPÄRC), 22. Jahrestagung der Vereinigung der Deutschen Ästhetisch-Plastischen Chirurgen (VDÄPC), 14th to 16th September, 2017 in Graz, Austria. Published in German Medical Science, DOI: 10.3205/17dgpraec258 .

Carolina Centeno-Cerdas, Montserrat Jarquin**,** Myra N. Chavez**,** Ursula Hopfner**,** Daniel Schmauss**,** Jörg Nickelsen**,** Matthias M. Aitzetmüller**,** Dominik Duscher**,** Hans-G. Machens**,** Hans Hauner**,** Tomas J. Egana. "Photosynthetic Stem Cell Therapy for Tissue Regeneration". Oral talk presented in the 48. Jahrestagung der Deutschen Gesellschaft der Plastischen, Rekonstruktiven und Ästhetischen Chirurgen (DGPRÄC), 55. Jahrestagung der Österreichischen Gesellschaft für Plastische, Ästhetische und Rekonstruktive Chirurgie (ÖGPÄRC), 22. Jahrestagung der Vereinigung der Deutschen Ästhetisch-Plastischen Chirurgen (VDÄPC), 14th to 16th September, 2017 in Graz, Austria. Published in German Medical Science, DOI: 10.3205/17dgpraec257

Matthias M. Aitzetmueller*, **Carolina Centeno Cerdas***, Phillipp Nessbach, Peter Foehr, Ursula Hopfner, Elizabeth Brett, Dominik Thor, Hans-Guenther Machens, Rainer Burgkart, Dominik Duscher. "PDO Threads for Facial Revjuvenation- Finding the Right Needle in the Haystack". Oral talk presented in the 8th Bozner Symposium of Plastic Surgery, 26th to 27th January 2018 in South Tyrol, Italy.

(*sharing first co-authorship).

Some other complementary work during the candidate's doctoral studies include:

Anna-Theresa Bauer, Katrin Lossagk, Matthias Aitzetmeuller, Ursula Hopfner, Manuela Kirsch, Daniel Schmauss, **Carolina Centeno**, Philipp Moog, Hans-Günther Machens, Dominik Duscher, Dominik von Lukowicz. Differentielle Genexpression in adipogenen Stammzellen und Adipozyten aus Lipödem und Kontrollfettgewebe. 49. Jahrestagung der Deutschen Gesellschaft der Plastischen, Rekonstruktiven und Ästhetischen Chirurgen (DGPRÄC), 23. Jahrestagung der Vereinigung der Deutschen Ästhetisch-Plastischen Chirurgen (VDÄPC). DOI: 10.3205/18dgpraec042

Matthias Aitzetmüller, P Nessbach, U Hopfner, M Kirsch, **C Centeno-Cerdas**, HG Machens, D Duscher. DFO preconditioning restores the therapeutic potential of diabetic ASCs. International Federation for Adipose Therapeutics and Science (IFATS). December 2017. https://ifats.memberclicks.net/.../IFATS_Program_Book_2017.pdf

M Aitzetmüller, P Nessbach, **C Centeno-Cerdas**, U Hopfner, M Kirsch, HG Machens, D Duscher. A fibrin spray system for the delivery of ASCs to diabetic wounds. International Federation for Adipose Therapeutics and Science (IFATS). December 2017. https://ifats.memberclicks.net/.../IFATS_Program_Book_2017.pdf

Morales-Sánches J.E, Calvo-Castro L.A., **Centeno-Cerdas C.**, Castro-Piedra S., Zeledón F., Guerrero M. and M. Rojas. 2015. Preliminary tests with stem cell therapies for the treatment of skin lesions in an animal model. Tissue Engineering: Part A. 21: S1-S413.

7. Manuscripts with first authorship

Development of photosynthetic sutures for the local delivery of oxygen and recombinant growth factors in wounds

Published in Acta Biomaterialia, ranked in the first quartil according to “Web of Science” and the “Scimago Journal and Country Rank”, with an impact factor of 6,383 and a 5-year impact factor of 7.160 (DOI: doi.org/10.1016/j.actbio.2018.09.060)

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Full length article

Development of photosynthetic sutures for the local delivery of oxygen and recombinant growth factors in wounds

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ABSTRACT

Surgical sutures represent the gold standard for wound closure, however, their main purpose is still limited to a mechanical function rather than playing a bioactive role. Since oxygen and pro-regenerative growth factors have been broadly described as key players for the healing process, in this study we evaluated the feasibility of generating photosynthetic sutures that, in addition to mechanical fixation, could locally and stably release oxygen and recombinant human growth factors (VEGF, PDGF-BB, or SDF-1α) at the wound site. Here, photosynthetic genetically modified microalgae were seeded in commercially available sutures and their distribution and proliferation capacity was evaluated. Additionally, the mechanical properties of seeded sutures were compared to unseeded controls that showed no significant differences. Oxygen production, as well as recombinant growth factor release was quantified *in vitro* over time, and confirmed that photosynthetic sutures are indeed a feasible approach for the local delivery of bioactive molecules. Finally, photosynthetic sutures were tested in order to evaluate their resistance to mechanical stress and freezing. Significant stability was observed in both conditions, and the feasibility of their use in the clinical practice was therefore confirmed. Our results suggest that photosynthetic gene therapy could be used to produce a new generation of bioactive sutures with improved healing capacities.

Statement of significance

Disruption of the vascular network is intrinsic to trauma and surgery, and consequently, wound healing is characterized by diminished levels of blood perfusion. Among all the blood components, oxygen and pro-regenerative growth factors have been broadly described as key players for the healing process. Therefore, in this study we evaluated the feasibility of generating photosynthetic sutures that, in addition to mechanical fixation, could locally and stably release oxygen and recombinant human growth factors at the wound site. This novel concept has never been explored before for this type of material and represents the first attempt to create a new generation of bioactive sutures with improved regenerative capabilities.

1. Introduction

Adult human wound healing is characterized by tissue repair rather than regeneration. In this regard, surgical procedures have evolved to become less invasive, and biomaterials more biocompatible, however, scarring still represents a major problem in modern surgery. This has deleterious consequences for the life-quality of patients, including mechanical [1–3], cosmetic [4,5], and economical aspects [6]. Moreover, in most cases scar tissue does not represent a functional tissue by itself, as it is mainly composed of extracellular matrix.

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Polydioxanone Threads for Facial Rejuvenation: Analysis of Quality Variation in the Market

Aitzetmueller, Matthias M. M.D.; Centeno Cerdas, Carolina M.Sc.; Nessbach, Phillipp M.Sc.; Foehr, Peter Dipl.-Ing.; Brett, Elizabeth M.Sc.; Thor, Dominik Ph.D.; Machens, Hans-Guenther M.D., Ph.D.; Burgkart, Rainer M.D., Ph.D.; Duscher, Dominik M.D., Ph.D.

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Abstract | Author Information | Article Metrics

Background: Beside botulinum-toxin injections and hyaluronic acid fillers, thread lifts have established themselves as the third column of minimally invasive facial rejuvenation. Most commonly, barbed threads for this approach are made out of polydioxanone, a material known for decades from application in resorbable sutures. The clinical efficacy and the putative material safety of polydioxanone have fueled the popularity of thread lifts.

Methods: The present study highlights significant variation among six commercially available threads in microstructure, tensile strength, elasticity, anchoring capacity in human tissue, and biocompatibility.

Results: Despite their license to be marketed and sold in the European Union, some products performed significantly worse than others on material testing, and even displayed cytotoxic characteristics.

Conclusion: The results of this study are highly relevant for clinicians and may be linked to various typical side effects of polydioxanone threads for facial rejuvenation.

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9. Affidavit

I hereby declare that the dissertation titled “Characterization and bioactivation of synthetic absorbable thread sutures for improved healing and cosmetic outcomes” prepared under the guidance and supervision of Univ.-Prof. Dr. Hans-Günther Machens, Univ.-Prof. Dr. Hans Hauner and Univ.-Prof. Dr. José Tomás Egaña-Erazo in the Plastic Surgery and Hand Surgery Department at Klinikum rechts der Isar, and submitted to the Medical Graduate Center of TUM is my own, original work undertaken in partial fulfillment of the requirements to obtain the doctoral degree. I have made no use of sources, materials or assistance other than those specified in § 6 (6) and (7), clause 2 of the “Regulations for the award of doctoral degrees” of TUM Graduate School.

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