Skin adhesion to the percutaneous component of direct bone anchored systems: Systematic review on preclinical approaches and biomaterials

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Nowadays, direct bone anchored systems are an increasingly adopted approach in the therapeutic landscape for amputee patients. However, the percutaneous nature of these devices poses a major challenge to obtain a stable and lasting proper adhesion between the implant surface and the skin. A systematic review was carried out in three databases (PubMed, Scopus, Web of Science) to provide an overview of the innovative strategies tested with preclinical models (*in vitro* and *in vivo*) in the last ten years to improve the skin adhesion of direct bone anchored systems. Fifty five articles were selected after screening, also employing PECO question and inclusion criteria. A modified Cochrane RoB 2.0 tool for the *in vitro* studies and the SYRCLE tool for in *in vivo* studies were used to assess the risk of bias. The evidence collected suggests that the implementation of porous percutaneous structures could be one of the most favorable approach to improve proper skin adhesion, especially in association with bioactive coatings, as hydroxyapatite, and exploiting the field of nanostructure. Some issues still remain open as (a) the identification and characterization of the best material/coating association able to limit the shear stresses at the interface and (b) the role of keratinocyte turnover on the skin/biomaterial adhesion and integration processes.

1. Introduction

Direct bone anchored systems have completely changed the therapeutic landscape for amputees. Amputation represents a traumatic psychological and social life-altering event with a huge impact on mobility, quality of life and daily routine. The leading cause of amputation, especially at the lower limb, is represented by dysvascular diseases and associated co-morbidities as diabetes (70%) followed by trauma (22%), tumors (4%), congenital deficiency and infection.[1,2] The standard of care for amputated patients is represented by custom socket prostheses that fit with the anatomy of the residual limb to allow the movements through the attached *exo*-prosthesis system and to transmit the forces from the skeleton to the ground.[3] Nevertheless, this type of approach is not suitable for all patients due to difficulties in achieving a proper socket fit and to complications as recurrent scarring, neuromas and fluctuations in the volume and/or length of the residual limb in patients with short residual limb. Frequent skin complications are represented by sores, ulcers, recurrent dermatitis and superficial infections thus severely limiting patient autonomy and the use of the prosthesis.[4] In 1999, Professor Per-Ingvar Brånemark carried out the first prospective study with a direct skeletal attachment system for the prosthesis to an amputated limb (OPRA-Osseointegrated Prostheses for the Rehabilitation of Amputees).[5,6] After OPRA, the interest and attention to this topic have increased considerably over time. Different direct skeletal attachment systems have been developed[7–13] thus suggesting the significance and the psychological/social impact of this therapeutic option. This is further corroborated by the FDA approvement in 2015 of the OPRA system for transfemoral amputation as Humanitarian Device Exemption (HDE).[14]

Complications related to the osteointegration of the intramedullary component with bone tissue have been reported.[8] On the other hand, the range of available solutions for bone integration due to the increased knowledge about this topic led to very good results in terms of osteointegration and made the management of the adverse events much more consolidated. Although the surgical implantation procedure of the direct skeletal attachment systems varies for type of implant,

design (geometry, porosity, surface modifications), surgical approach, and rehabilitation protocol, the creation of a percutaneous access point to connect the intramedullary component with the *exo*-prosthesisis necessary. As for other permanent percutaneous devices (*i.e.*, bone anchored hearing devices, dental implants,), one of the main drawback is represented by the creation of a skin or mucosal penetration site.[15,16] Firmly and stable epithelial cell adhesion to implant surface is fundamental to prevent the development of infections. Superficial infections at the skin/implant interface represent one of the most frequently reported complication with a rate ranging from 18–41% *Staphylococcus aureus* and coagulase-negative Staphylococci are the most common detected bacteria.[8,17,96] Breaching the skin with a permanent implant could lead to the formation of a sinus tract referred as "*marsupialization phenomenon*" or epidermal downgrowth "*permigration*" with pocket formation if a properly skin sealing is not achieved. Within these pockets, waste material from cell turnover, liquids, *etc*. are collected so representing a fertile ground for bacterial growth and proliferation. To the best of author's knowledge, the development of a strategy able to ensure the proper adhesion of the skin and soft tissues to the implant surfaces is still an unsolved issue. This major topic is crucial for the complete success of this approach.

Starting from clinical literature on bone anchored systems, we deeply investigated the skin/implant components of these devices, exploring the preclinical literature on innovative strategies under investigation, the progress of these researches and the obtained evidences to translate preclinical improvements to the clinical application. Iin light of the increasing use of these direct bone anchored systems. The aim of our study is (i) to review the solutions specifically proposed for percutaneous devices in direct bone anchored system for amputees to provide "*a state of the art*" about strategies, approaches and materials under evaluation; (ii) to understand which approaches may be of clinical interest and translation from bench to bedsite and finally (iii) to identify aspects still to be defined, elucidated or taken into consideration to really achieve a lasting skin/implant adhesion.

2. Materials and methods

2.1. Eligibility criteria

In order to be included in the following systematic review, the population of interest (P), exposure (E), comparators and outcomes (CO) were established, formulating the PECO statement. The Population considered in this review focused on preclinical *in vitro* and *in vivo* studies which object was the skin component in direct skeletal connection systems. Studies focused on percutaneous components of the bone anchored hearing devices and dental studies have been excluded. Despite some common features, important differences exist in embryologic development, structure, blood supply, mechanical forces, cutaneous and muscular components, microenvironment and bone forming and remodelling capabilities between craniofacial and appendicular bone with particular reference to arms and legs.[18] *In vitro* studies included human or animal cell lines or primary cells from the skin tissue as well as co-cultures and three-dimensional models (3D). *In vivo* studies included all animal models in which a percutaneous/ transcutaneous implant was performed, with an extensive analysis of the cutaneous compartment. The Exposure item considered in this review was any new type of material, coating, surface functionalization or modification strategy or therapeutic approach adopted to promote skin, cells or tissue, adhesion to the percutaneous/transcutaneous implant. The Comparator was any reference group (cells or animals) not exposed to the innovative approach (material, coating, functionalization, modification and therapy). The primary Outcomes for the *in vitro* studies included were (1) adhesion, (2) viability, (3) proliferation. The secondary considered outcomes were the execution of (1) molecular investigations or (2) immunocytochemistry to evaluate cell differentiation. The considered primary outcomes for the *in vivo* models were (1) the adhesion of the dermal and subdermal tissue to the implant surface and subsequently (2) the presence or absence of epidermal downgrowth and its quantitative/qualitative evaluation from the histological point of view and finally (3) the histological analysis of all tissues surrounding the percutaneous/ transcutaneous

implant. In addition, the considered secondary outcomes were (1) immunohistochemical characterization of the regenerated tissue, as important information that can be obtained from antibody phenotype expression especially for keratinocytes with a different stage of maturation and differentiation and (2) microbiological investigations. Review, editorials, commentaries, conference abstract, studies published in other language than English were excluded as well as clinical studies. All this information has been summarized in Table 1.

2.2. Search strategy

The literature search was performed according to the PRISMA guidelines and approach tools.[19] Electronic searching was performed within 3 databases including published researches in English language, full text only restriction and ranging date from 2010–2020. The selected databases were PubMed (http:// www.ncbi.nlm.nih.gov/pubmed), Scopus (https://www.scopus. com) and ISI Web of Knowledge (https://apps.webofknowledge. com). To enhance the database search sensitivity and specificity the combination of the following terms "percutaneous implant", "percutaneous device", "transcutaneous implant", "transcutaneous device", "skin integration" and "skin sealing" was searched as keywords or MeSH-terms with boolean operators (AND, OR) in each database, as reported in detailed search strategies provided as ESI in Appendix A. Titles and abstracts were evaluated first and then submitted to Zotero (version 5.0.94) to eliminate duplicates and managing the references. Subsequently, the full-text assessment of the remaining articles was performed. Reference lists of each retrieved article was also screened to identify any additional relevant study for the topic of the review. Eligibility of the articles was evaluated independently and in duplicate by two authors (MS and MM), while disagreements were resolved through discussion even reaching a consensus with the involvement of a third reviewer (MF). The flowchart depicted in Fig. 1 summarizes the process.

2.3. Parameters extracted from the articles

The following parameters were extracted from *in vitro* papers:

(1) tested materials; (2) cell type; (3) performed analyses and experimental times; (4) main outcomes and (5) main conclusions. The retrieved information from *in vivo* papers referred to (1) experimental design; (2) materials/coatings; (3) performed evaluations; (4) main outcomes and (5) main conclusions. All the extracted information has been summarized and tabulated in ESI Tables 1 and 2, respectively.

2.4. Risk of bias assessment within individual studies

Risk of Bias assessment was performed by two authors, independently (MS and MM). Since there is no validated tool to assess the Risk of Bias for *in vitro* studies, the updated Cochrane RoB 2.0 tool[20] was specifically modified to try to assess this potential risk. The assessed criteria included "selection bias", "performance bias", "attrition bias" and "reporting issues".[21] The SYRCLE risk of bias tool was used for papers based on *in vivo* studies. Assessed criteria were "selection bias", "performance bias", "detection bias", "attrition bias", "reporting bias", and "other bias".[22]

3. Results

The literature search retrieved 543 citations: 356 from PubMed database, 15 from Scopus and 172 from Web of Science. The records after duplicate removal were 526 and after the screening of the title and abstract, 53 articles were reviewed to evaluate their inherence with PECO question and inclusion criteria. After this further screening *35* articles were recognized eligible for the review and from the references list, 20 additional articles were added, not found in the starting literature search. Thus, a total of *55* articles were included and analyzed, of which *23* were *in vitro*, *28 in vivo* and 4 both *in vitro* and *in vivo*. The selection procedure is reported in Fig. 1 in agreement with the PRISMA methodological tool. The major findings were briefly summarized in Table 2 while an extensive data tabulation have been reported in ESI Tables 1 and 2† on *in vitro* and *in*

146 *vivo* studies, respectively. The shared references between *vitro* and *vivo* were highlighted with a mark
147 (*). In addition, in Appendix B a ESI table† with the PRISMA checklist was reported.

148 **3.1. *In vitro* risk of bias assessment**

149 The quality of evidence provided by the *in vitro* and *in vivo* studies was evaluated adopting the
150 Cochrane RoB 2.0 tool modified to be applied to *in vitro* studies (Fig. 2) and the SYRCLE's risk of
151 bias for the *in vivo* studies (Fig. 3). Although the *in vitro* studies represent a fundamental step in the
152 translational decision-making process and in obtaining and consolidating evidences, there are no
153 specific guidelines for quality assessment. The RoB 2.0 tool was then adapted to include elements
154 useful in the identification of the potential biases (*i.e.*, selection bias, performance bias, attrition
155 bias and reporting bias). For the selection bias, intended as baseline similarities between the
156 experimental groups/conditions, the bias was judged as "low" in almost all studies. In fact, an
157 accurate explanation about sample preparation and handling was reported helping the reader to
158 understand the experimental set-up. It was not possible to assign a risk for blinding outcome
159 assessment (detection bias) due to the lack of this information in the texts (unclear judgment).
160 Incomplete outcome data (attrition bias) in term of evidence of experimental groups or conditions
161 loss or excluded without clear explanation, implies a "high" RoB. In the same way, outcomes
162 stated in the experimental set-up but not reported in the result section were judged at "high" risk.
163 The last parameter for the *in vitro* RoB refers to "other source of bias" detected by reader in the
164 overall reported study as statistical analysis not clearly reported or not appropriate.
165 Summarizing, the retrieved *in vitro* studies reported in a detailed way the experimental setup
166 adopted to evaluate materials/coatings/strategies/ approaches. The source of high risk is related to
167 the attrition and reporting biases as in some studies the reported findings were not adequately
168 supported by the methodologies or by the results described in the text.

169 **3.2. *In vivo* risk of bias assessment**

170 In order to evaluate the biases in the included *in vivo* studies, SYRCLE's risk of bias tool was

adopted (Fig. 3). Most studies reported the use of a randomization process but failed to provide details about the used methodology. Also, the details about the allocation concealment and blinding were rather scarce or unclear in the author's opinion. Beyond these aspects, it was easy for the reader to follow the flow of the animals throughout the study as no particularly numerous experimental groups characterized the studies. The defined outcomes were very similar among the studies and the failure in evaluating a specimen or the exclusion of an animal from the studies was duly justified. Rigorous reporting of the data was not always respected and "problems that could result in high risk of bias" were detected in the studies with few animals in which relevant treatment effects might not be detected and false negative error (type II error) may be produced.

Thus, the retrieved *in vivo* studies about the percutaneous implant model for orthopedic applications seem to be mostly affected by biases in randomization and blinding field of application. These biases then affects many other items of this tool in which blinding plays a central role.

3.3. Narrative results synthesis

***3.3.1. In vitro* studies.** Detailed information about the study design and main outcomes of included studies has been reported in ESI Table 1.† *In vitro* studies are wide-ranging and in some specific cases very innovative.[23–49] The cell types predominantly used are fibroblasts of human origin in 12 studies[23–34] and animal in 9 studies.[35–43] Human keratinocytes were used in 10 studies[24,26,28,31,35,44–48] and one study was performed using cells from animal origin.[49] Five studies were performed analyzing both cell types,[24,26,28,31,35] while the other studies were focused on fibroblasts or keratinocytes. A 3D model was adopted in one study[23] and a co-culture with fibroblasts and keratinocytes was used in another one.[26] In the remaining *in vitro* studies, the set-up was based on the direct contact of a cell monolayer with materials or coating surfaces.

As previously mentioned, several innovative approaches resulted from this literature search. Concerning new emerging technologies, the additive manufacturing melt electrowriting

technique (MEW) was adopted by E. C. L. Bolle *et al.*, to print a poly(ε-caprolactone) porous polymer structure in nano– micrometer range able to promote adhesion and colonization of seeded fibroblasts, thus representing a suitable approach to modified the surface of percutaneous devices.[23] Micro arc oxidation (MAO) is an electrochemical surface approach to generate a microporous and adherent coating of TiO_2. The high potential of MAO approach is used to produce discharges that penetrate the oxide coating allowing the doping with ions present in the electrolytic solution as copper (Cu) or zinc (Zn) in order to confer also antibacterial properties to the coating.[36–39] The transfer of antibacterial properties to the coating has been studied not only exploiting Cu and Zn which are relatively new in association with percutaneous devices, but also by making coatings with nanostructured silver (Ag) content to inhibit biofilm formation on percutaneous surfaces.[32,33,46] MAO technique was also combined with the surface functionalization with Fe_3O_4 magnetic nanoparticles to exploit the magnetic field gradient to further enhance fibroblast cell response and to also explore the unknown bacterial killing effect mediated by the production of reactive oxygen species by the Fe_3O_4 nanoparticles exposure.[40] MAO was also studied in combination with hydrothermal treatment (HT) to modify the topography, obtaining features as nanoplates, nanorods and nanofibers, or the chemical composition of the surfaces.

As expected, a great interest has been devoted to the nanostructure. In the present review, 13 studies are focused on the production of nanostructured features by adopting different approaches as alkali heat treatment alone[25] or combined with HT,[41] anodization[26,31,50] electron beam evaporation,[45,48] microwave plasma chemical vapor.[47] The nanofeatures, generally imposed onto metallic implants, improved the surface area thanks to a greater nanoscale roughness. As a consequence, a greater availability of reactive sites to target the basic process of protein adsorption, concentration and conformation is generated so determining an improvement in cell adhesion, spreading and proliferation.

Another important line of research is represented by surface functionalization with organic

molecules. In these papers key molecules for skin tissue are investigated *i.e.*, transmembrane proteins that mediate the interaction to the actin cytoskeleton as E-Cadherin,[24,49] or extracellular matrix proteins as laminin 332 or fibronectin (Fn)[27,28,30] with an essential involvement in cell motility and epithelial migration and organization. Fibroblast growth factor 2 (FGF-2) has been studied in association with nanostructured surfaces and a significant improvement in keratinocyte density after 5 days of culture was detected in comparison to conventional titanium[48] or fibroblast control cultures.[42] A. Trent *et al*., explored the use of keratose or keratine extracted from human hair fibers to realize a nano-keratin coating on titanium surfaces (as humans do not possess the specific enzyme keratinases to degrade these proteins) to realize a resilient coating for percutaneous device.[28] Indeed, fibronectin, a fundamental glycoprotein that mediates cell interaction acting at different level of complexity, has been extensively investigated for the percutaneous surface functionalization. Different chemical approaches to overcome the desorption process that could occur during *in vivo* implantation have also been studied as silanization of the surfaces or passivation.[27,28,30]

Finally, a surface coating with bioactive ceramic materials has been exploited, first of all adopting hydroxyapatite (HA) but also fluoroapatite, fluorohydroxypatite,[25] octacalcium phosphate,[42] HA substituted with ionic species as silicon,[41] observing an enhanced response *in vitro* in terms of cell adhesion and proliferation.

3.3.2. ***In vivo* studies.** Thirty-two *in vivo* studies have been performed by using different animal models (ESI Table 2†): 7 studies on sheep,[27,51–55] 7 studies in mice,[40,41,56–60] 7 rats,[42,61–66] 4 studies in rabbits,[26,45,67,68] 3 studies in guinea pigs[69–71] and 2 studies inn swines/micro-pigs.[72,73] One study adopted a feline model[74] and another one reported the clinical and histological data concerning 4 dogs which had undergone amputations due to tumors treated with direct skeletal attachment osteointegrated system.[75] The present search provides a framework in which 3 type of percutaneous implantation types have been adopted: (1) amputation of a limb and subsequent implantation of the intramedullary, percutaneous and *exo*-prosthesis components in 6 studies; (2)

subcutaneous implantation in the dorsum or scapulae, but also in the scalp with the implant partially protruding outside in 15 studies, and finally (3) back implant in which the skin is pierced in a through-and-through fashion, to obtain two exit sites, generally used to study polymeric materials (6 studies out of 32).

Three additional studies with a tibia percutaneous implant have been evaluated.[52,54,68] Two studies without the presence of the percutaneous component were also included in the review: one study with the subcutaneous implant in the tibia of plates coated with adsorbed or silanized fibronectin in comparison with control surfaces and coatings[27] and another study with the implantation in the paraspinal muscles.[51] The latter well investigated the influence of implant porosity as the combination of different porosity dimension and strut size and the evaluation of soft tissue penetration and colonization.[51]

In most of the evaluated papers, the devices were made of titanium (23 studies) and medical grade Ti6Al4V alloy (14 studies), when specified. The evaluation of different combinations of porosities and structures from the histological and histomorphometric point of views have been investigated in different preclinical studies, as the optimal porosity range that could positively favour the colonization of soft tissues and limit the downgrowth phenomenon has not yet been identified with certainty. Some studies were focused on percutaneous implants coated with the commercial porous titanium coating type “P2” or “K2” in comparison with smooth polished surfaces. These studies well describe and elucidate the process of cell infiltration, neovascularization, epidermal downgrowth and formation of a fibrous capsule induced by these types of coating already used to coat the subdermal flange component of osteointegrated implants in clinical applications[53,55,62,65,69–71,76]

Compared to *in vitro* preclinical studies, the nanostructure seems to be less in the spotlight, since among the studies included in the present research 4 papers out of 32 have been specifically devoted to the investigations on nanostructured surfaces obtained with anodization, alkali heat, hydrothermal treatment or MAO.[26,40,41,61] The histological results suggest a better performance of

for the nanostructured surfaces in comparison to the polished titanium ones both in terms of integration and reduction of epithelial downgrowth and of antibacterial activity.[26] Also *in vivo*, HA coatings and surface functionalization with Fn, HAFn of HAFnAg, laminin 332, have been evaluated as *in vivo* translation of promising results previously obtained with *in vitro* studies.[27,51,57,66] The investigation of antimicrobial agents appears to be less represented: E. L. Perry *et al.*, evaluated the antimicrobial properties of CSA-13, a cationic steroid compound derived from bile acids and loaded on polyurethane foam pads and placed in direct contact with the skin at the exit site of a percutaneous tibial implant,[54] reporting it was not effective in preventing infections. H. Qu *et al.*, experimented a sol–gel film to release triclosan (2,4,40-trichloro-20-hydroxydiphenylether) from a percutaneous tibial pin surface[68] and found no signs of infection around implants coated with sol–gel film also detecting tissue normal healing and reduced epithelial downgrowth.

Finally, differently from the *in vitro* findings, polymers seems to be of considerable interest for the percutaneous component. Polymers, natural or synthetic or a combination of both, represent the most frequently biomaterials adopted for skin regeneration thanks to biocompatibility properties and similarity to the skin (natural polymers) and good controllable mechanical and physical properties (synthetic polymers).[76] The results and considerations reported in the retrieved papers provide interest insights that could help in developing better performing materials or surface coatings. The research activities focused on naturally porous poly(2-hydroxyethyl methacrylate) [poly(HEMA)], an hydrogel used for soft contact lens, evaluated different pore size and interconnecting throat configuration, as it is or functionalized with an adhesive agent (carbonyldiimidazole, CDI) or lamin332 or a combination of both. The performed studies aimed to elucidate the skin regeneration and integration inside the polymer structure from the morphological and compositional point of view[56,57] as elasticity properties of this device are close to those of the skin one, thus representing a possible candidate for percutaneous coating. A. Oyane *et al.*, and K. Sasaki *et al.*, focused their investigation on ethylene-vinyl alcohol copolymer (EVOH) coated with HA or with laminin[42] or FGF-2[64] respectively immobilized into

HA. In both studies the immobilization of the organic molecules improved the adhesion between the polymer film and the skin reducing the downgrowth and without compromising the HA biocompatibility. Finally, P. Pholpabu *et al.*, investigated an elastomeric matrix of poly (glycerol-*co*-sebacate)-cinnamate (PGS-cinA) loaded with lipopolysaccharide (LPS) or linoleic acid (LA) or a combination of both detecting and increase in M2 macrophage population, a reduction of skin contraction and attenuation in epidermal downgrowth when implanted percutaneously in mouse dorsal skin.[59]

Negative Pressure Wound Therapy (NPWT) was evaluated in different animal models (rats and guinea pigs) and the analyzed studies reported that NWPT was useful in reducing the edematous reaction and the downgrowth phenomenon, favouring vascularization and also exerting a favorable mechanical action on tissues.[69–71] Nevertheless, a drawback has been highlighted by D. R. L. Pawar *et al.*, who found that NWPT beneficial effects were present only if the treatment was used continuously. In fact, a NWPT discontinuation led to results comparable to the untreated control.[71]

4. Discussion

The translation of the osteointegration concepts from its original field of application to direct bone anchored implant systems, has been implementing for 20 years but it still represents a very challenging topic. One of the main "hot spot" in these osteointegrated types of implants is represented by the percutaneous access that create a permanent breach in the skin barrier, leaving the implant vulnerable to infection development if a stable seal is not achieved between implant surface and skin. Percutaneous access has always represented a complicated topic and already in the 80s von Recum claimed the "*percutaneous device dilemma*" referring to devices as central venous catheters, peritoneal dialysis catheters, ventricular assist devices.[16] But the clinical applications of percutaneous devices have been expanded and increased especially with the advent of a "new" type of percutaneous device represented by the direct skeletal attachment systems for

amputated patients or bone conduction hearing aid in which a percutaneous device permanently protrude through the skin connecting an *exo*-prosthesis to the bone tissue. For the integration of these specific devices with the bone tissue huge literature data and great experience is available with reported good results. Different and limited is the knowledge on skin integration and related improvement strategies.

Thus, the present review focused on the interaction of the skin component with the percutaneous device to summarize the state of the art about this specific topic and to provide a general vision about the critical points and the strengths of the new approaches and strategies developed to overcome the *percutaneous device dilemma.*

To the best author's knowledge and despite several preclinical researches, none of the proposed approaches has been actually translated to the clinical practice related to the direct bone anchored systems for amputee patients. The skin/ implant interface is mainly managed with the use of polished, smooth titanium. This surface probably minimizes skin irritation and inflammation. It is also reported that epithelial cells prefer to growth on smooth rather than on rough surfaces. But evidences on the influence that topography exerts on epithelial cells are still conflicting. Only the Intraosseous Transcutaneous Amputation Prosthesis (ITAP) system adopted an hydroxyapatite coating for the percutaneous component, but no specific details are available after the completion of the associated clinical trial.[77] However the breaking down of the hydroxyapatite interface is reported so leading to implant failure and infection.[78] Even if the translation from bench to bedside is not particularly strong at the moment for the skin/ implant interface component, there are some promising approaches. For example, the surface functionalization with organic proteins seems to favour the adhesion of the epithelial cells both *in vitro* than *in vivo* as key molecules of epidermal and dermal components are usually exploited, specifically involved in cell adhesion and recognition pathways. Nevertheless, this type of approach may face with the degree of adsorption on the surface or the efficiency of linking mechanism and subsequent *in vivo* degradability of these molecules. Probably the surface functionalization with organic molecules

could be used in custom or personalized approaches, in particularly complicated conditions, once defined the effective lasting of this type of coating.

Widely exploitable and translatable approaches are those based on nanostructured surfaces and nanostructured polymer or ceramic coatings. The coating with ceramics by means of electrochemical or plasma spray deposition, the creation of a nanostructured topography with alkali-heat or hydrothermal treatment, micro-arc oxidation or anodization could represent a clinically translable strategy realized with the adoption of well accessible technologies. Furthermore, the effectiveness of the deposition of ceramic coatings could be easily and quickly verifiable as well as the degradation profiles are largely known for almost all ceramic materials adopted in the biomedical field. Likewise, also nanostructured surfaces which seem to favour the adhesion and growth of fibroblasts and keratinocytes, could be quite easy manufactured and the characterization of the physical–chemical properties of the coatings are consolidated procedures.

The *in vitro* studies provide useful insights for the improvement of skin sealing around the percutaneous post as those derived from studies focused on nanostructure surfaces. In fact, the summary of the main outcomes retrieved from the analyzed papers demonstrated improved cellular response in term of adhesion, proliferation, differentiation and migration with nanometer surfaces probably because the nanostructure significantly impacts on the initial phase of protein absorption thanks to the increased surface energy, hydrophilicity and roughness.[79]

However, in the author's opinion several limitations exist that should be considered in the experimental design of future studies about this topic regarding the *in vitro* studies. Bidimensional (2D) cultures with one cell type certainly provide useful information on the possible cell behavior but do not closely resemble the *in vivo* processes as 3D models in which structural, biochemical and biomechanical interactions recreate more realistically the microenvironment complexity that occur *in vivo*.[80] Cell–cell interaction as well as cell–matrix interactions in 3D models better reflect the *in vivo* real condition as they prompt cells commitment to differentiation developing different functional conditions and subsets. This aspect is crucial for skin which is a tissue made up of

highly specialized and multilayered cells subjected to a constant renewal in its outermost layer. In fact, the dead keratinocytes, lost by the desquamation process in stratum corneum, are constantly replaced by new cells produced in the basal layer. These new cells travel upward, pushing existing older cells in the "keratinization process" which lasts about 4 weeks, according with age, genetic and environmental factors.[81]

Only one paper out of 27 adopted advanced models, in which a reconstructed human skin equivalent models (HSE) was used to evaluate a polymeric scaffold made with melt electrowriting techniques.[23] The HSE model was used to investigate the fibroblasts behavior on the scaffold and the scaffold mechanical integration with a pull-out *in vitro* test. In experimenting new coatings, scaffolds or strategies, it should be taken into greater consideration that the skin is a very specialized viscoelastic tissue able to respond to a wide range of mechanical cues thanks to the intracellular signaling pathways that convert these stimuli into biochemical responses by means of complex mechanoresponsive and transductive elements.[82]

The lack of an *in vitro* multy-layered 3D models able to reproduce the keratinocyte's turnover represents an important limitation for the translation of findings.

The most easily translatable evidence certainly comes from the *in vivo* studies in which the experimental design is mainly focused on the identification and evaluation of the effect of titanium with different range of porosities on the epidermal downgrowth phenomenon in association with tissue penetration, colonization and differentiation. The conclusions that can be drawn about the optimal porosity range are not univocal, as the animal models and experimental set-ups are very different from each other, starting from the choice of the site of implant. Limb amputation model comprises the mechanical load component thus allowing more speculative conclusions. These studies seem to suggest that titanium porosity ranging between 30–50% for the subdermal component with a pore size of about 100 μm, could be able to limit skin regression and the marsupialization phenomenon for several months after implant.[50,53,73,74] Thus, greater attention to the mechanical cues would be expected from *in vivo* studies. Nevertheless, most of the implant

sites are not subjected to mechanical load: the through-and-through fashion and percutaneous implant partially protruding onto the back of animals are reported in 20 out of 32 studies. Three additional papers were retrieved with transverse implant approach in animal tibia in which the load is different from the axial load of the amputation model, but certainly more representative of a clinical condition and less demanding from an ethical point of view.[52,54,68]

For example, the comprehension of shear stress influences as well as the mechanical discontinuity that accumulates at the skin/implant interface represent a crucial point that could influence the healing process. In this view, an adopted strategy in transcutaneous implant has been the introduction of subdermal flanges with holes or porous subdermal barriers as a biomimetic approach that resembles a natural transcutaneous structure represented by the deer antler. These particular structures composed by porous subcutaneous bone tissue, with a mean porosity of 217 μm (±19.07 μm), favour the colonization by dermis and epidermidis ensuring a strong seal between the bone/skin interface.[83] Moreover, this percutaneous structure is represented in humans by the teeth, hence the Prof. Brånemark intuition to translate the principles of dental implant osteointegration also to the amputee's patients. In reproducing the same approach, the problems seem to be greater and the degree of success very variable. It is speculated by several authors that the main problems potentially come from the greater soft tissue coverage around limb implants, which increases the interfacial movement. Fibroblasts of the oral cavity have a more rapid proliferation and extracellular matrix organizational capability. In addition, the expression of several cytokines and growth factors is higher in comparison to what observed for dermal fibroblasts.[33]

Flanges or subcutaneous barriers, adopted to increase the adhesion surface for fibroblasts and keratinocytes, seem to provide a major stabilization of the soft tissue and to reduce the movement of the epithelium at the interface thus reducing the downgrowth.[55,74,75] The results obtained by S. Jeyapalina *et al.*, with P2 porous-coated subdermal barrier seem to demonstrate a reduction in superficial infection around the exit site of implant and marsupialization phenomenon. Nine

429 months after one-stage surgical implant, the migration in smooth subdermal barrier was 2.1
430 micrometer in comparison to 0.6 micrometer obtained with the subdermal porous coating.[53] E. L.
431 Perry *et al.*, performing a percutaneous implant at the level of tibia metaphysis, stressed how the
432 excessive skin mobility and the presence of subcutaneous tissue led to the development of
433 infections in 19 animals out of 20.[54] Also, computational model seems to suggest that the use of
434 approaches that dissipate the stresses at the interface through the stabilization of the soft tissue
435 could significantly improve the outcomes thanks to skin adhesion and the reduction of breakdown
436 phenomena that can also elicit and increase of local inflammation.[84] Nevertheless, recently
437 published evidence by the same research group has resized the effect of the subdermal barrier: in
438 evaluating the longer-term efficacy of porous subdermal barrier up to 24 months, a progressive
439 epithelial downgrowth was detected of about 0.343 ± 0.07 mm per month with a 17% of infection
440 development.[76]
441 In the authors' opinion limited attention has been paid to the immune system component.
442 Immunohistochemical evaluations of the macrophage and lymphocyte population have been
443 reported but mainly linked to the verification of the inflammatory status or infective events. No
444 studies investigating deeply the role of the immune system in this complex external/internal
445 interaction have been found. Yet the role played by the immune system could represent an
446 important element that caouldalso influence epithelial cell migration as suggested by R.
447 A. Underwood *et al.*,[58] as well as the vascular component: a properly perfused skin could target
448 components of the immune system influencing in agonistic but also antagonistic way epithelial
449 cells migration.[85,86] In addition, the immune system plays a fundamental role in the delicate
450 interaction with the skin microbiota and an increasing attention is now devoted to this balancing.[87]
451 Several investigations have been performed comparing the skin and the abutment of bone
452 anchored hearing systems. Different microbial species have been found when comparing the intact
453 skin with the breached skin site. Moreover, an inflammatory state influences the microbiological
454 profile at the abutment skin site.[88] Also the variation in the molecular expression of anabolic and

455 catabolic cytokines has been reported even in the absence of visible inflammation signs so
456 suggesting the constant balance and activation of immune system.[89] In this view, a longitudinal
457 cohort study recently published, focused on patients with direct bone anchored systems for
458 transfemoral amputation. The microbiological profile was analysed from the molecular point of
459 view at the time of surgery up to one year after the second-stage surgery. DNA bacterial extraction
460 from swab was performed at the stoma, at the intact adjacent skin of the operated leg and from the
461 contralateral thigh skin. The obtained findings suggested that a different microbiota characterized
462 the adjacent thigh skin and the skin of the contralateral thigh from the stomal microbiota in which
463 a significant relevant presence of *Staphylococcus aureus* was detected. The microbiological profile
464 at the stoma was not stable and shifted over time with the selection of communities represented by
465 *Streptococcus*, *Corynebacterium*, and/or *Staphylococcus* spp.[90] Therefore, the knowledge of
466 microbiota and its changes could help the understanding on conditions favoring healthy skin and
467 predisposing to the development of an infection to target therapies to the pathogens responsible for
468 the inflammatory/infectious state.
469 Another factor that is not taken into consideration, in *in vivo* preclinical studies, is the factor
470 related to the daily hygiene of the stoma. It is reported that in the clinical practice patients are
471 encouraged to perform a daily hygiene of the stoma cleaning the site with soap and water or with
472 specific cleaning tool as dental oxyjet.[91] Some of the preclinical approaches evaluated in this
473 review seems to be promising and also translable to the clinical scenario, but the impact that a
474 daily hygiene practice can exert on the surface of the percutaneous component (*i.e.*, nanostructured
475 or polymer-coated), is still to be evaluated. In all the above reported considerations, it is always
476 necessary to remember that substantial differences exist between *in vivo* models and human
477 skin.[92] For example, sheep have a greater density of hair follicles, the presence of the wool and
478 glands secretions as lanolin which performs a water-repellent function, or the expression of growth
479 factors related to the seasonality of the wool itself. Some animals as mice or rats are characterized
480 by *panniculus carnosus*, a thin layer of subcutaneous striated muscle attached to the skin and

fascia, virtually absent in the human skin, which allows the independent movement of the skin with respect to the deeper tissues.[93] In addition, metacarpus, where the amputation has been performed in sheep models is devoid of musculature and hypodermis which reduces the edematous reaction, instead very frequent in the post-operative phase in humans. Again, in animal models as rat, mouse or rabbit the primary healing mechanism consists in the contraction while in humans' skin, healing proceeds through a re-epithelization process.[94] Actually, the animal model quite similar for human skin studies is represented by the pig, but some important factors could limit its use (purchase and maintenance).[95]

5. Conclusion

According to the evidences collected from the synthesis of the *in vitro* and *in vivo* studies and based on the effective translation, it is author's opinion that working on porous percutaneous structures, further implementing the use of flanges or subdermal barriers, seems to be one of the most advantageous and favorable approach to improve skin adhesion. The use of HA coatings, especially if nanostructured, seems to be similarly positive in terms of outcome for skin adhesion as well as the use of polymer coatings with mechanical properties more similar to those of the native skin.

In conclusion some basic aspects still remain to be elucidated that could represent the turning point in this specific as (i) a deeper understanding of the of skin healing process in presence of permanently implanted biomaterial; (ii) the identification and characterization of the best compromise materials/coatings in which the mechanical properties closely match to those of the skin to limit the shear stresses at the interface and (iii) the role of keratinocytes turnover on the skin/biomaterial adhesion and integration process.

Conflicts of interest

There are no conflicts to declare.

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References

1 K. Ziegler-Graham, E. J. MacKenzie, P. L. Ephraim, T. G. Travison and R. Brookmeyer, Estimating the prevalence of limb loss in the United States: 2005 to 2050, *Arch. Phys. Med. Rehabil.*, 2008, 89(3), 422–429, DOI: 10.1016/j.apmr.2007.11.005, PMID: 18295618.

2 P. Varma, M. G. Stineman and T. R. Dillingham, Epidemiology of limb loss, *Phys. Med. Rehabil. Clin. North Am.*, 2014, 25(1), 1–8, DOI: 10.1016/j.pmr.2013.09.001, PMID: 24287235; PMCID: PMC4533906.

3 K. Hagberg and R. Brånemark, Consequences of non-vascular trans-femoral amputation: a survey of quality of life, prosthetic use and problems, *Prosthet. Orthot. Int.*, 2001, 25(3), 186–194, DOI: 10.1080/03093640108726601, PMID:11860092.

4 J. S. Hebert, M. Rehani and R. Stiegelmar, Osseointegration for Lower-Limb Amputation: A Systematic Review of Clinical Outcomes, *JBJS Rev.*, 2017, 5(10), e10, DOI: 10.2106/JBJS.RVW.17.00037, PMID: 29087966.

5 R. Brånemark, P. I. Brånemark, B. Rydevik and R. R. Myers, Osseointegration in skeletal reconstruction and rehabilitation: a review, *J. Rehabil. Res. Dev.*, 2001, 38(2), 175–181. PMID: 11392650.

6 K. Hagberg, R. Brånemark, B. Gunterberg and B. Rydevik, Osseointegrated trans-femoral amputation prostheses: prospective results of general and condition-specific quality of life in 18 patients at 2-year follow-up, *Prosthet. Orthot. Int.*, 2008, 32(1), 29–41, DOI: 10.1080/03093640701553922, PMID: 18330803.

7 H. H. Aschoff, R. E. Kennon, J. M. Keggi and L. E. Rubin, Transcutaneous, distal femoral, intramedullary attachment for above-the-knee prostheses: an endo-exo device, *J. Bone Jt. Surg., Am. Vol.*, 2010, 92(Suppl 2), 180–186, DOI: 10.2106/JBJS.J.00806, PMID: 21123601.

8 M. A. Muderis, K. Tetsworth, A. Khemka, S. Wilmot, B. Bosley, S. J. Lord and V. Glatt, The Osseointegration Group of Australia Accelerated Protocol (OGAAP-1) for twostage osseointegrated

reconstruction of amputated limbs, *Bone Jt. J.*, 2016, 98-B(7), 952–960, DOI: 10.1302/0301620X.98B7.37547, PMID: 27365474.

9 Early Feasibility Study of the Percutaneous Osseointegrated Prosthesis (POP)— http://clinicaltrials.gov/ct2/show/study/ NCT02720159.

10 L. Guirao, B. Samitier, R. Tibau, J. Alós, M. Monago, M. Morales-Suarez-Varela and E. Pleguezuelos, Distance and speed of walking in individuals with trans-femoral amputation fitted with a distal weight-bearing implant, *Orthop. Traumatol. Surg. Res.*, 2018, 104(6), 929–933, DOI: 10.1016/j.otsr.2018.04.011, Epub 2018 Jun 1. PMID: 29864519.

11 R. L. McGough, M. A. Goodman, R. L. Randall, J. A. Forsberg, B. K. Potter and B. Lindsey, The Compress® transcutaneous implant for rehabilitation following limb amputation, *Unfallchirurg*, 2017, 120(4), 300–305, DOI: 10.1007/s00113-017-0339-9, English, PMID: 28275847.

12 Intraosseous transcutaneous amputation prosthesis- https://clinicaltrials.gov/ct2/show/NCT02491424?term= ITAP&rank=1.

13 A. Thesleff, R. Brånemark, B. Håkansson and M. OrtizCatalan, Biomechanical Characterisation of Bone-anchored Implant Systems for Amputation Limb Prostheses: A Systematic Review, *Ann. Biomed. Eng.*, 2018, 46(3), 377–391, DOI: 10.1007/s10439-017-1976-4, Epub 2018 Jan 11. PMID: 29327257; PMCID: PMC5809556.

14 G. Liu, E. Chen, D. Lewis and G. Rao, Food and Drug Administration's Humanitarian Device Exemption Marketing Approval Pathway: Insights for Developing Devices for Rare Diseases, *J. Med. Devices*, 2017, 11(3), 034701, DOI: 10.1115/1.4036333.

15 A. Peramo and C. L. Marcelo, Bioengineering the skin implant interface: the use of regenerative therapies in implanted devices, *Ann. Biomed. Eng.*, 2010, 38(6), 2013–2031, DOI: 10.1007/s10439-010-9937-1, Epub 2010 Feb 6. PMID: 20140520.

16 K. Affeld, J. Grosshauser, L. Goubergrits and U. Kertzscher, Percutaneous devices: a review of

applications, problems and possible solutions, *Expert Rev. Med. Devices*, 2012, 9(4), 389–399, DOI: 10.1586/erd.12.25, PMID: 22905843.

17 J. Tillander, K. Hagberg, L. Hagberg and R. Brånemark, Osseointegrated titanium implants for limb prostheses attachments: infectious complications, *Clin. Orthop. Relat. Res.*, 2010, 468(10), 2781–2788, DOI: 10.1007/s11999-010-1370-0, Epub 2010 May 15. PMID: 20473597; PMCID: PMC2939339.

18 D. Wang, J. R. Gilbert, X. Zhang, B. Zhao, D. F. E. Ker and G. M. Cooper, Calvarial Versus Long Bone: Implications for Tailoring Skeletal Tissue Engineering, *Tissue Eng., Part B*, 2020, 26(1), 46–63, DOI: 10.1089/ten.TEB.2018.0353, Epub 2019 Dec 18. PMID: 31588853.

19 M. J. Page, J. E. McKenzie, P. M. Bossuyt, I. Boutron, T. C. Hoffmann, C. D. Mulrow, L. Shamseer, J. M. Tetzlaff, E. A. Akl, S. E. Brennan, R. Chou, J. Glanville, J. M. Grimshaw, A. Hróbjartsson, M. M. Lalu, T. Li, E. W. Loder, E. Mayo-Wilson, S. McDonald, L. A. McGuinness, L. A. Stewart, J. Thomas, A. C. Tricco, V. A. Welch, P. Whiting and D. Moher, The PRISMA 2020 statement: an updated guideline for reporting systematic reviews, *Br. Med. J.*, 2021, 372, n71, DOI: 10.1136/bmj.n71, PMID: 33782057; PMCID: PMC8005924.

20 J. A. C. Sterne, J. Savović, M. J. Page, R. G. Elbers, S. Blencowe, I. Boutron, C. J. Cates, H. Y. Cheng, M. S. Corbett, S. M. Eldridge, J. R. Emberson, M. A. Hernán, S. Hopewell, A. Hróbjartsson, D. R. Junqueira, P. Jüni, J. J. Kirkham, T. Lasserson, T. Li, A. McAleenan, B. C. Reeves, S. Shepperd, I. Shrier, L. A. Stewart, K. Tilling, I. R. White, P. F. Whiting and J. P. T. Higgins, RoB 2: a revised tool for assessing risk of bias in randomised trials, *Br. Med. J.*, 2019, 366, l4898, DOI: 10.1136/bmj.l4898, PMID: 31462531.

21 A. Iliadi, D. Koletsi, S. N. Papageorgiou and T. Eliades, Safety Considerations for Thermoplastic-Type Appliances Used as Orthodontic Aligners or Retainers. A Systematic Review and Meta-Analysis of Clinical and In-Vitro Research, *Materials*, 2020, 13(8), 1843, DOI: 10.3390/ma13081843, PMID: 32295303; PMCID: PMC7215465.

22 C. R. Hooijmans, M. M. Rovers, R. B. de Vries, M. Leenaars, M. Ritskes-Hoitinga and M. W. Langendam, SYRCLE's risk of bias tool for animal studies, *BMC Med. Res. Methodol.*, 2014, 14, 43, DOI: 10.1186/1471-2288-14-43, PMID: 24667063; PMCID: PMC4230647.

23 E. C. L. Bolle, N. Bartnikowski, P. Haridas, T. J. Parker, J. F. Fraser, S. D. Gregory and T. R. Dargaville, Improving skin integration around long-term percutaneous devices using fibrous scaffolds in a reconstructed human skin equivalent model, *J. Biomed. Mater. Res., Part B*, 2020, 108(3), 738–749, DOI: 10.1002/jbm.b.34428, Epub 2019 Jun 6. PMID: 31169980.

24 J. Dehli, C. Karlsson, C. Bizelli-Silveira, X. Jiang, D. Kraft and M. Foss, E-cadherin mediated cell-biomaterial interaction reduces migration of keratinocytes in-vitro, *Colloids Surf., B*, 2019, 180, 326–333, DOI: 10.1016/j.col-surfb.2019.04.010, Epub 2019 Apr 5. PMID: 31075686.

25 M. Yamada, E. Kato, A. Yamamoto and K. Sakurai, A titanium surface with nano-ordered spikes and pores enhances human dermal fibroblastic extracellular matrix production and integration of collagen fibers, *Biomed. Mater.*, 2016, 11(1), 015010, DOI: 10.1088/1748-6041/11/1/015010, PMID: 26835848.

26 J. Tan, C. Zhao, J. Zhou, K. Duan, J. Wang, X. Lu, J. Weng and B. Feng, Co-culturing epidermal keratinocytes and dermal fibroblasts on nano-structured titanium surfaces, *Mater. Sci. Eng., C*, 2017, 78, 288–295, DOI:10.1016/j.msec.2017.04.036, Epub 2017 Apr 7. PMID:28575986.

27 M. Chimutengwende-Gordon, C. Pendegrass and G. Blunn, Enhancing the soft tissue seal around intraosseous transcutaneous amputation prostheses using silanized fibronectin titanium alloy, *Biomed. Mater.*, 2011, 6(2), 025008, DOI: 10.1088/1748-6041/6/2/025008, Epub 2011 Feb 28. PMID: 21358026.

28 A. Trent and M. E. Van Dyke, Development and characterization of a biomimetic coating for percutaneous devices, *Colloids Surf., B*, 2019, 182, 110351, DOI: 10.1016/j.colsurfb.2019.110351, Epub 2019 Jul 8. PMID: 31326624.

614 29 C. J. Pendegrass, C. A. Middleton, D. Gordon, J. Jacob and G. W. Blunn, Measuring the strength
615 of dermal fibroblast attachment to functionalized titanium alloys in vitro, *J. Biomed.*
616 *Mater. Res., Part A*, 2010, 92(3), 1028–1037, DOI: 10.1002/jbm.a.32450, PMID: 19296546.
617 30 C. J. Pendegrass, M. El-Husseiny and G. W. Blunn, The development of fibronectin-
618 functionalised hydroxyapatite coatings to improve dermal fibroblast attachment in vitro, *Bone Jt.*
619 *Surg., Br. Vol.*, 2012, 94(4), 564–569, DOI: 10.1302/0301-620X.94B4.27698, PMID: 22434476.
620 31 B. S. Smith, S. Yoriya, T. Johnson and K. C. Popat, Dermal fibroblast and epidermal
621 keratinocyte functionality on titania nanotube arrays, *Acta Biomater.*, 2011, 7(6), 2686–2696,
622 DOI: 10.1016/j.actbio.2011.03.014, Epub 2011 Mar 15. PMID: 21414425.
623 32 J. Song, S. J. Remmers, J. Shao, E. Kolwijck, X. F. Walboomers, J. A. Jansen, S. C. Leeuwenburgh
624 and F. Yang, Antibacterial effects of electrospun chitosan/poly (ethylene oxide) nanofibrous
625 membranes loaded with chlorhexidine and silver, *Nanomedicine*, 2016, 12(5), 1357–1364, DOI:
626 10.1016/j.nano.2016.02.005, Epub 2016 Mar 10. PMID: 26970025.
627 33 M. Chimutengwende-Gordon, C. Pendegrass, R. Bayston and G. Blunn, Preventing infection of
628 osseointegrated transcutaneous implants: Incorporation of silver into preconditioned fibronectin-
629 functionalized hydroxyapatite coatings suppresses Staphylococcus aureus colonization while
630 promoting viable fibroblast growth in vitro, *Biointerphases*, 2014, 9(3), 031010, DOI:
631 10.1116/1.4889977, PMID: 25280851.
632 34 T. Calliess, M. Sluszniak, A. Winkel, C. Pfaffenroth, W. Dempwolf, W. Heuer, H.
633 Menzel, H. Windhagen and M. Stiesch, Antimicrobial surface coatings for a permanent
634 percutaneous passage in the concept of osseointegrated extremity prosthesis, *Biomed. Tech.*,
635 2012, 57(6), 467–471, DOI: 10.1515/bmt-2011-0041, PMID: 23183722.
636 35 B. T. Bennett, J. P. Beck, K. Papangkorn, J. S. Colombo, K. N. Bachus, J. Agarwal, J. F.
637 Shieh and S. Jeyapalina, Characterization and evaluation of fluoridated apatites for the
638 development of infection-free percutaneous devices, *Mater. Sci. Eng., C*, 2019, 100, 665–

675, DOI: 10.1016/j.msec.2019.03.025, Epub 2019 Mar 12. PMID: 30948103.

36 L. Zhang, Y. Han and G. Tan, Hydroxyaptite nanorods patterned ZrO2 bilayer coating on zirconium for the application of percutaneous implants, *Colloids Surf., B*, 2015, 127, 8–14, DOI: 10.1016/j.colsurfb.2015.01.017, Epub 2015 Jan 17. PMID: 25635615.

37 L. Zhang, J. Guo, X. Huang, Y. Zhang and Y. Han, The dual function of Cu-doped TiO2 coatings on titanium for application in percutaneous implants, *J. Mater. Chem. B*, 2016, 4(21), 3788–3800, DOI: 10.1039/c6tb00563b, Epub 2016 May 6. PMID: 32263317.

38 L. Zhang, J. Zhang, F. Dai and Y. Han, Cytocompatibility and antibacterial activity of nanostructured H2Ti5O11·H_2O outlayered Zn-doped TiO2 coatings on Ti for percutaneous implants, *Sci. Rep.*, 2017, 7(1), 13951, DOI: 10.1038/s41598017-13954-4, PMID: 29066726; PMCID: PMC5654996.

39 L. Zhang, J. Guo, T. Yan and Y. Han, Fibroblast responses and antibacterial activity of Cu and Zn co-doped TiO 2 for percutaneous implants, *Appl. Surf. Sci.*, 2018, 434, 633–642, DOI: 10.1016/j.apsusc.2017.10.169.

40 K. Li, S. Liu, Y. Xue, L. Zhang and Y. Han, A superparamagnetic Fe3O4-TiO2 composite coating on titanium by micro-arc oxidation for percutaneous implants, *J. Mater. Chem. B*, 2019, 7(34), 5265–5276, DOI: 10.1039/c9tb01096c, Epub 2019 Aug 6. PMID: 31384861.

41 K. Li, Y. Xue, T. Yan, L. Zhang and Y. Han, Si substituted hydroxyapatite nanorods on Ti for percutaneous implants, *Bioact. Mater.*, 2020, 5(1), 116–123, DOI: 10.1016/j.bioactmat.2020.01.001, PMID: 32021946; PMCID: PMC6994265.

42 K. Sasaki, A. Oyane, K. Hyodo, A. Ito, Y. Sogo, M. Kamitakahara and K. Ioku, Preparation and biological evaluation of a fibroblast growth factor-2-apatite composite layer on polymeric material, *Biomed. Mater.*, 2010, 5(6), 065008, DOI: 10.1088/1748-6041/5/6/065008, Epub 2010 Oct 22. PMID: 20966534.

43 M. A. Shevtsov, O. V. Galibin, N. M. Yudintceva, M. I. Blinova, G. P. Pinaev, A. A. Ivanova, O. N.

664 Savchenko, D. N. Suslov, I. L. Potokin, E. Pitkin, G. Raykhtsaum and M. R. Pitkin, Twostage
665 implantation of the skin- and bone-integrated pylon seeded with autologous fibroblasts
666 induced into osteoblast differentiation for direct skeletal attachment of limb prostheses: TS
667 Implantation of the SBIP., *J. Biomed. Mater. Res., Part A*, 2014, 102(9), 3033–3048, DOI:
668 10.1002/jbm.a.34969.
669 44 D. J. Gordon, D. D. Bhagawati, C. J. Pendegrass, C. A. Middleton and G. W. Blunn,
670 Modification of titanium alloy surfaces for percutaneous implants by covalently attaching laminin, *J.*
671 *Biomed. Mater. Res., Part A*, 2010, 94(2), 586–593, DOI: 10.1002/jbm.a.32735, PMID: 20198691.
672 45 S. D. Puckett, P. P. Lee, D. M. Ciombor, R. K. Aaron and T. J. Webster, Nanotextured titanium
673 surfaces for enhancing skin growth on transcutaneous osseointegrated devices, *Acta Biomater.*,
674 2010, 6(6), 2352–2362, DOI: 10.1016/j.actbio.2009.12.016, Epub 2009 Dec 11. PMID:
675 20005310.
676 46 S. K. Mishra, S. Raveendran, J. M. Ferreira and S. Kannan, In Situ Impregnation of Silver
677 Nanoclusters in Microporous Chitosan-PEG Membranes as an Antibacterial and Drug Delivery
678 Percutaneous Device, *Langmuir*, 2016, 32(40), 10305–10316, DOI: 10.1021/acs.langmuir.6b02844,
679 Epub 2016 Sep 29. PMID: 27685160.
680 47 S. A. Skoog, A. V. Sumant, N. A. Monteiro-Riviere and R. J. Narayan, Ultrananocrystalline
681 Diamond-Coated Microporous Silicon Nitride Membranes for Medical Implant Applications,
682 *JOM*, 2012, 64, 520–525, DOI: 10.1007/s11837-012-0300-x.
683 48 M. A. Zile, S. Puckett and T. J. Webster, Nanostructured titanium promotes keratinocyte density, *J.*
684 *Biomed. Mater. Res., Part A*, 2011, 97(1), 59–65, DOI: 10.1002/jbm.a.33028, Epub 2011 Feb 11.
685 PMID: 21319293.
686 49 C. J. Pendegrass, B. Tucker, S. Patel, R. Dowling and G. W. Blunn, The effect of adherens
687 junction components on keratinocyte adhesion in vitro: potential implications for sealing the
688 skin-implant interface of intraosseous transcutaneous amputation prostheses, *J. Biomed. Mater. Res.,*

689 *Part A*, 2012, 100(12), 3463–3471, DOI: 10.1002/jbm.a.0, Epub 2012 Jul 20. PMID:
690 22815157.

691 50 M. A. Shevtsov, N. Yudintceva, M. Blinova, G. Pinaev, Galibin, I. Potokin, K. C. Popat and M.
692 Pitkin, Application of the skin and bone integrated pylon with titanium oxide nanotubes and seeded
693 with dermal fibroblasts, *Prosthet. Orthot. Int.*, 2015, 39(6), 477–486, DOI:
694 10.1177/0309364614550261, Epub 2014 Sep 23. PMID: 25249382; PMCID: PMC4370813.

695 51 M. Chimutengwende-Gordon, R. Dowling, C. Pendegrass and G. Blunn, Determining the
696 porous structure for optimal soft-tissue ingrowth: An in vivo histological study, *PLoS One*,
697 2018, 13(10), e0206228, DOI: 10.1371/journal. pone.0206228, PMID: 30372471; PMCID:
698 PMC6205611.

699 52 M. Chimutengwende-Gordon, C. Pendegrass and G. Blunn, The in vivo effect of a porous titanium
700 alloy flange with hydroxyapatite, silver and fibronectin coatings on softtissue integration of
701 intraosseous transcutaneous amputation prostheses, *Bone Jt. J.*, 2017, 99-B(3), 393–400, DOI:
702 10.1302/0301-620X.99B3.BJJ-2016-0360.R1, PMID: 28249981; PMCID: PMC5358203.

703 53 S. Jeyapalina, J. P. Beck, K. N. Bachus, D. L. Williams and R. D. Bloebaum, Efficacy of a
704 porous-structured titanium subdermal barrier for preventing infection in percutaneous
705 osseointegrated prostheses, *J. Orthop. Res.*, 2012, 30(8), 1304–1311, DOI: 10.1002/jor.22081,
706 Epub 2012 Jan 31. PMID: 22294380.

707 54 E. L. Perry, J. P. Beck, D. L. Williams and R. D. Bloebaum, Assessing peri-implant tissue infection
708 prevention in a percutaneous model, *J. Biomed. Mater. Res., Part B*, 2010, 92(2), 397–408, DOI:
709 10.1002/jbm.b.31528, PMID: 19927331.

710 55 B. M. Holt, K. N. Bachus, J. P. Beck, R. D. Bloebaum and S. Jeyapalina, Immediate post-
711 implantation skin immobilization decreases skin regression around percutaneous osseointegrated
712 prosthetic implant systems, *J. Biomed. Mater. Res., Part A*, 2013, 101(7), 2075–2082, DOI:
713 10.1002/jbm.a.34510, Epub 2012 Dec 27. PMID: 23281016.

56 P. Fleckman, M. Usui, G. Zhao, R. Underwood, M. Maginness, A. Marshall, C. Glaister, B. Ratner and J. Olerud, Cutaneous and inflammatory response to longterm percutaneous implants of sphere-templated porous/ solid poly(HEMA) and silicone in mice, *J. Biomed. Mater. Res., Part A*, 2012, 100(5), 1256–1268, DOI: 10.1002/jbm.a.34012, Epub 2012 Feb 23. PMID: 22359383; PMCID: PMC3506026.

57 Y. Fukano, M. L. Usui, R. A. Underwood, S. Isenhath, A. J. Marshall, K. D. Hauch, B. D. Ratner, J. E. Olerud and Fleckman, Epidermal and dermal integration into sphere-templated porous poly(2-hydroxyethyl methacrylate) implants in mice, *J. Biomed. Mater. Res., Part A*, 2010, 94(4), 1172–1186, DOI: 10.1002/jbm.a.32798, PMID: 20694984; PMCID: PMC2998188.

58 R. A. Underwood, M. L. Usui, G. Zhao, K. D. Hauch, M. M. Takeno, B. D. Ratner, A. J. Marshall, X. Shi, J. E. Olerud and P. Fleckman, Quantifying the effect of pore size and surface treatment on epidermal incorporation into percutaneously implanted sphere-templated porous biomaterials in mice, *J. Biomed. Mater. Res., Part A*, 2011, 98(4), 499–508, DOI: 10.1002/jbm.a.33125, Epub 2011 Jun 16. PMID: 21681942; PMCID: PMC4684638.

59 P. Pholpabu, S. S. Yerneni, C. Zhu, P. G. Campbell and C. J. Bettinger, Controlled Release of Small Molecules from Elastomers for Reducing Epidermal Downgrowth in Percutaneous Devices, *ACS Biomater. Sci. Eng.*, 2016, 2(9), 1464–1470, DOI: 10.1021/acsbiomaterials.6b00192, Epub 2016 Aug 18. PMID: 33440584.

60 T. Calliess, I. Bartsch, M. Haupt, M. Reebmann, M. Schwarze, M. Stiesch, C. Pfaffenroth, M. Sluszniak, W. Dempwolf, H. Menzel, F. Witte and E. Willbold, In vivo comparative study of tissue reaction to bare and antimicrobial polymer coated transcutaneous implants, *Mater. Sci. Eng., C*, 2016, 61, 712–719, DOI: 10.1016/j.msec.2015.12.095, Epub 2016 Jan 4. PMID: 26838901.

61 B. J. Farrell, B. I. Prilutsky, J. M. Ritter, S. Kelley, K. Popat and M. Pitkin, Effects of pore size, implantation time, and nano-surface properties on rat skin ingrowth into percutaneous porous

titanium implants, *J. Biomed. Mater. Res., Part A*, 2014, 102(5), 1305–1315, DOI: 10.1002/jbm.a.34807, Epub 2013 Jun 7. PMID: 23703928; PMCID: PMC3871976.

62 D. Isackson, K. J. Cook, L. D. McGill and K. N. Bachus, Mesenchymal stem cells increase collagen infiltration and improve wound healing response to porous titanium percutaneous implants, *Med. Eng. Phys.*, 2013, 35(6), 743–753, DOI: 10.1016/j.medengphy.2012.08.002, Epub 2012 Aug 31. PMID: 22940446; PMCID: PMC3529992.

63 K. Nam, R. Matsushima, T. Kimura, T. Fujisato and A. Kishida, In vivo characterization of a decellularized dermis-polymer complex for use in percutaneous devices, *Artif. Organs*, 2014, 38(12), 1060–1065, DOI: 10.1111/aor.12330, Epub 2014 Jun 25. PMID: 24962020.

64 A. Oyane, K. Hyodo, M. Uchida, Y. Sogo and A. Ito, Preliminary in vivo study of apatite and laminin-apatite composite layers on polymeric percutaneous implants, *J. Biomed. Mater. Res., Part B*, 2011, 97(1), 96–104, DOI: 10.1002/jbm.b.31790, Epub 2011 Feb 2. PMID: 21290587.

65 D. R. L. Pawar, S. Jeyapalina, K. Hafer and K. N. Bachus, Influence of negative pressure wound therapy on peri-prosthetic tissue vascularization and inflammation around porous titanium percutaneous devices, *J. Biomed. Mater. Res., Part B*, 2019, 107(6), 2091–2101 DOI: 10.1002/jbm.b.34302, Epub 2019 Jan 10. PMID: 30629801.

66 S. Asoda, T. Arita and K. Takakuda, Mechanical attachment of soft tissue to dental and maxillofacial implants with mesh structures: an experiment in percutaneous model, *J. Biomed. Mater. Res., Part B*, 2013, 101(4), 553–559, DOI: 10.1002/jbm.b.32855, Epub 2012 Dec 20. PMID: 23281217.

67 D. Isackson, L. D. McGill and K. N. Bachus, Percutaneous implants with porous titanium dermal barriers: an in vivo evaluation of infection risk, *Med. Eng. Phys.*, 2011, 33(4), 418–426, DOI: 10.1016/j.medengphy.2010.11.007, Epub 2010 Dec 10. PMID: 21145778; PMCID: PMC3071885.

68 H. Qu, C. Knabe, S. Radin, J. Garino and P. Ducheyne, Percutaneous external fixator pins with bactericidal micron-thin sol-gel films for the prevention of pin tract infection, *Biomaterials*,

764 2015, 62, 95–105, DOI: 10.1016/j.biomaterials.2015.05.041, Epub 2015 May 27. PMID:
765 26036176.
766 69 S. J. Mitchell, S. Jeyapalina, F. R. Nichols, J. Agarwal and K. N. Bachus, Negative pressure
767 wound therapy limits downgrowth in percutaneous devices, *Wound Repair. Regen.*, 2016, 24(1),
768 35–44, DOI: 10.1111/wrr.12373, Epub 2015 Dec 2. PMID: 26487170; PMCID: PMC4805517.
769 70 S. Jeyapalina, S. J. Mitchell, J. Agarwal and K. N. Bachus, Biomimetic coatings and negative
770 pressure wound therapy independently limit epithelial downgrowth around percutaneous devices,
771 *J. Mater. Sci.: Mater. Med.*, 2019, 30(6), 71, DOI: 10.1007/s10856-019-6272-4, PMID: 31183809.
772 71 D. R. L. Pawar, S. J. Mitchell, S. Jeyapalina, J. E. Hawkes, S. R. Florell and K. N. Bachus, Peri-
773 prosthetic tissue reaction to discontinuation of negative pressure wound therapy around porous
774 titanium percutaneous devices, *J. Biomed. Mater. Res., Part B*, 2019, 107(3), 564–572, DOI:
775 10.1002/jbm.b.34148, Epub 2018 May 7. PMID: 29732684.
776 72 R. Hugate, R. Clarke, T. Hoeman and A. Friedman, Transcutaneous Implants in a Porcine Model:
777 The Use of Highly Porous Tantalum, *Int. J. Adv. Mater. Res.*, 2015, 1(2), 32–40.
778 73 B. M. Holt, D. H. Betz, T. A. Ford, J. P. Beck, R. D. Bloebaum and S. Jeyapalina, Pig dorsum
779 model for examining impaired wound healing at the skin-implant interface of percutaneous
780 devices, *J. Mater. Sci.: Mater. Med.*, 2013, 24(9), 2181–2193, DOI: 10.1007/s10856-013-4975-5,
781 Epub 2013 Jul 6. PMID: 23832453; PMCID: PMC3770289.
782 74 B. J. Farrell, B. I. Prilutsky, R. S. Kistenberg, J. F. Dalton 4th and M. Pitkin, An animal model to
783 evaluate skin-implantbone integration and gait with a prosthesis directly attached to the residual
784 limb, *Clin. Biomech.*, 2014, 29(3), 336–349, DOI: 10.1016/j.clinbiomech.2013.12.014, Epub 2013
785 Dec 23. PMID: 24405567; PMCID: PMC3959271.
786 75 N. Fitzpatrick, T. J. Smith, C. J. Pendegrass, R. Yeadon, M. Ring, A. E. Goodship and G. W.
787 Blunn, Intraosseous transcutaneous amputation prosthesis (ITAP) for limb salvage in 4 dogs, *Vet.*
788 *Surg.*, 2011, 40(8), 909–925, DOI: 10.1111/j.1532-950X.2011.00891.x, Epub 2011 Nov 4. PMID:

789 22092391.
790 76 M. Sheikholeslam, M. E. E. Wright, M. G. Jeschke and S. Amini-Nik, Biomaterials for Skin
791 Substitutes, *Adv. Healthcare Mater.*, 2018, 7(5), DOI: 10.1002/adhm.201700897,
792 Epub 2017 Dec 22. PMID: 29271580; PMCID: PMC7863571.
793 77 S. Jeyapalina, J. P. Beck, J. Agarwal and K. N. Bachus, A 24-month evaluation of a
794 percutaneous osseointegrated limb-skin interface in an ovine amputation model, *J. Mater. Sci.:*
795 *Mater. Med.*, 2017, 28(11), 179, DOI: 10.1007/s10856-017-5980-x, PMID: 28980174.
796 78 J. S. Hoellwarth, K. Tetsworth, S. R. Rozbruch, M. B. Handal, A. Coughlan and M. Al
797 Muderis, Osseointegration for Amputees: Current Implants, Techniques, and Future Directions,
798 *JBJS Rev.*, 2020, 8(3), e0043, DOI: 10.2106/JBJS.RVW.19.00043, PMID: 32224634; PMCID:
799 PMC7161721.
800 79 M. Jäger, H. P. Jennissen, F. Dittrich, A. Fischer and H. L. Köhling, Antimicrobial and
801 Osseointegration Properties of Nanostructured Titanium Orthopaedic Implants, *Materials*,
802 2017, 10(11), 1302, DOI: 10.3390/ ma10111302, PMID: 29137166; PMCID: PMC5706249.
803 80 M. J. Randall, A. Jüngel, M. Rimann and K. Wuertz-Kozak, Advances in the Biofabrication of 3D
804 Skin in vitro: Healthy and Pathological Models, *Front. Bioeng. Biotechnol.*, 2018, 6, 154, DOI:
805 10.3389/fbioe.2018.00154, PMID: 30430109; PMCID: PMC6220074.
806 81 H. Niehues, J. A. Bouwstra, A. El Ghalbzouri, J. M. Brandner, P. L. J. M. Zeeuwen
807 and E. H. van den Bogaard, . 3D skin models for 3R research: The potential of 3D reconstructed
808 skin models to study skin barrier function, *Exp. Dermatol.*, 2018, 27(5), 501–511, DOI:
809 10.1111/exd.13531, Epub 2018 Apr 6. PMID: 29518287.
810 82 V. W. Wong, S. Akaishi, M. T. Longaker and G. C. Gurtner, Pushing back: wound
811 mechanotransduction in repair and regeneration, *J. Invest. Dermatol.*, 2011, 131(11), 2186–2196,
812 DOI: 10.1038/jid.2011.212, Epub 2011 Jul 21. PMID: 21776006.
813 83 C. J. Pendegrass, A. E. Goodship, J. S. Price and G. W. Blunn, Nature's answer to

breaching the skin barrier: an innovative development for amputees, *J. Anat.*, 2006, 209(1), 59–67, DOI: 10.1111/j.1469-7580.2006.00595.x, PMID: 16822270; PMCID: PMC2100310.

84 S. Yerneni, Y. Dhaher and T. A. Kuiken, A computational model for stress reduction at the skin-implant interface of osseointegrated prostheses, *J. Biomed. Mater. Res., Part A*, 2012, 100(4), 911–917, DOI: 10.1002/jbm.a.34028, Epub 2012 Jan 24. PMID: 22275149.

85 J. A. S. Quaresma, Organization of the Skin Immune System and Compartmentalized Immune Responses in Infectious Diseases, *Clin. Microbiol. Rev.*, 2019, 32(4), e00034–e00018, DOI: 10.1128/CMR.00034-18, PMID: 31366611; PMCID: PMC6750136.

86 A. V. Nguyen and A. M. Soulika, The Dynamics of the Skin's Immune System, *Int. J. Mol. Sci.*, 2019, 20(8), 1811, DOI: 10.3390/ijms20081811, PMID: 31013709; PMCID: PMC6515324.

87 M. Coates, S. Blanchard and A. S. MacLeod, Innate antimicrobial immunity in the skin: A protective barrier against bacteria, viruses, and fungi, *PLoS Pathog.*, 2018, 14(12), e1007353, DOI: 10.1371/journal.ppat.1007353, PMID: 30522130; PMCID: PMC6283644.

88 T. G. A. Calon, M. Trobos, M. L. Johansson, J. van Tongeren, M. van der Lugt-Degen, A. M. L. Janssen, P. H. M. Savelkoul, R. J. Stokroos and A. E. Budding, Microbiome on the Bone-Anchored Hearing System: A Prospective Study, *Front. Microbiol.*, 2019, 10, 799, DOI: 10.3389/fmicb.2019.00799, PMID: 31105654; PMCID: PMC6498861.

89 T. G. A. Calon, J. van Tongeren, O. Omar, M. L. Johansson and R. J. Stokroos, Cytokine expression profile in the boneanchored hearing system: 12-week results from a prospective randomized, controlled study, *Clin. Implant. Dent. Relat. Res.*, 2018, 20(4), 606–616, DOI: 10.1111/cid.12615, Epub 2018 Apr 27. PMID: 29701288; PMCID: PMC6099213.

90 J. P. Beck, M. Grogan, B. T. Bennett, S. Jeyapalina, J. Agarwal, C. Bartow-McKenney, J. Bugayev, E. Kubiak, S. Sinclair and E. Grice, Analysis of the Stomal Microbiota of a Percutaneous Osseointegrated Prosthesis: A Longitudinal Prospective Cohort Study, *J. Orthop. Res.*, 2019, 37(12), 2645–2654, DOI: 10.1002/jor.24421, Epub 2019 Aug 2. PMID: 31317568.

91 https://www.osseointegration.eu/stoma-care.

92 H. D. Zomer and A. G. Trentin, Skin wound healing in humans and mice: Challenges in translational research, *J. Dermatol. Sci.*, 2018, 90(1), 3–12, DOI: 10.1016/j.jdermsci.2017.12.009, Epub 2017 Dec 26. PMID: 29289417.

93 N. Naldaiz-Gastesi, O. A. Bahri, A. López de Munain, K. J. A. McCullagh and A. Izeta, The panniculus carnosus muscle: an evolutionary enigma at the intersection of distinct research fields, *J. Anat.*, 2018, 233(3), 275–288, DOI: 10.1111/joa.12840, Epub ahead of print. PMID: 29893024; PMCID: PMC6081499.

94 L. Rittié, Cellular mechanisms of skin repair in humans and other mammals, *J. Cell Commun. Signal.*, 2016, 10(2), 103–120, DOI: 10.1007/s12079-016-0330-1, Epub 2016 May 12. PMID: 27170326; PMCID: PMC4882309.

95 A. Grada, J. Mervis and V. Falanga, Research Techniques Made Simple: Animal Models of Wound Healing, *J. Invest. Dermatol.*, 2018, 138(10), 2095–2105, DOI: 10.1016/j.jid.2018.08.005, PMID: 30244718.

96 M. Al Muderis, A. Khemka, S. J. Lord, H. Van de Meent and J. P. M. Frölke, Safety of Osseointegrated Implants for Transfemoral Amputees: A Two-Center Prospective Cohort Study, *The Journal of Bone and Joint Surgery, American Volume*, 2016, 98(11), 900–909, DOI: 10.2106/JBJS.15.00808.