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1 **Skin adhesion to the percutaneous component of direct bone anchored**
2 **systems: systematic review on preclinical approaches and biomaterials**

3 Maria Sartori, Veronica Borsari, Melania Maglio, Silvia Brogini, Laura Bragonzoni, Stefano Zaffagnini and
4 Milena Fini

5

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

20

21 **1. Introduction**

22 Direct bone anchored systems have completely changed the therapeutic landscape for amputees.
23 Amputation represents a traumatic psychological and social life-altering event with a huge impact
24 on mobility, quality of life and daily routine. The leading cause of amputation, especially at the
25 lower limb, is represented by dysvascular diseases and associated co-morbidities as diabetes (70%)
26 followed by trauma (22%), tumors (4%), congenital deficiency and infection.^{1,2} The standard of
27 care for amputated patients is represented by custom socket prostheses that fit with the anatomy of
28 the residual limb to allow the movements through the attached *exo*-prosthesis system and to
29 transmit the forces from the skeleton to the ground.³ Nevertheless, this type of approach is not
30 suitable for all patients due to difficulties in achieving a proper socket fit and to complications as
31 recurrent scarring, neuromas and fluctuations in the volume and/or length of the residual limb in
32 patients with short residual limb. Frequent skin complications are represented by sores, ulcers,
33 recurrent dermatitis and superficial infections thus severely limiting patient autonomy and the use of
34 the prosthesis.⁴ In 1999, Professor Per-Ingvar Brånemark carried out the first prospective study with a
35 direct skeletal attachment system for the prosthesis to an amputated limb (OPRA-Osseointegrated
36 Prostheses for the Rehabilitation of Amputees).^{5,6} After OPRA, the interest and attention to this topic
37 have increased considerably over time. Different direct skeletal attachment systems have been
38 developed⁷⁻¹³ thus suggesting the significance and the psychological/social impact of this
39 therapeutic option. This is further corroborated by the FDA approval in 2015 of the OPRA
40 system for transfemoral amputation as Humanitarian Device Exemption (HDE).¹⁴
41 Complications related to the osteointegration of the intramedullary component with bone tissue
42 have been reported.⁸ On the other hand, the range of available solutions for bone integration due
43 to the increased knowledge about this topic led to very good results in terms of osteointegration
44 and made the management of the adverse events much more consolidated. Although the surgical
45 implantation procedure of the direct skeletal attachment systems varies for type of implant,

46 design (geometry, porosity, surface modifications), surgical approach, and rehabilitation protocol,
47 the creation of a percutaneous access point to connect the intramedullary component with the *exo-*
48 prosthesis necessary. As for other permanent percutaneous devices (*i.e.*, bone anchored hearing
49 devices, dental implants,), one of the main drawback is represented by the creation of a skin or
50 mucosal penetration site.^{15,16} Firmly and stable epithelial cell adhesion to implant surface is
51 fundamental to prevent the development of infections. Superficial infections at the skin/implant
52 interface represent one of the most frequently reported complication with a rate ranging from 18–
53 41% *Staphylococcus aureus* and coagulase-negative Staphylococci are the most common detected
54 bacteria.^{8,17,96} Breaching the skin with a permanent implant could lead to the formation of a sinus
55 tract referred as “*marsupialization phenomenon*” or epidermal downgrowth “*permigration*” with
56 pocket formation if a properly skin sealing is not achieved. Within these pockets, waste material
57 from cell turnover, liquids, *etc.* are collected so representing a fertile ground for bacterial growth
58 and proliferation. To the best of author’s knowledge, the development of a strategy able to ensure
59 the proper adhesion of the skin and soft tissues to the implant surfaces is still an unsolved issue.
60 This major topic is crucial for the complete success of this approach.

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

72 2. Materials and methods

73 2.1. Eligibility criteria

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

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

103 **2.2. Search strategy**

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

120 **2.3. Parameters extracted from the articles**

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

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

127 **2.4. Risk of bias assessment within individual studies**

128 Risk of Bias assessment was performed by two authors, independently (MS and MM). Since
129 there is no validated tool to assess the Risk of Bias for *in vitro* studies, the updated Cochrane RoB
130 2.0 tool²⁰ was specifically modified to try to assess this potential risk. The assessed criteria
131 included “selection bias”, “performance bias”, “attrition bias” and “reporting issues”.²¹ The
132 SYRCLE risk of bias tool was used for papers based on *in vivo* studies. Assessed criteria were
133 “selection bias”, “performance bias”, “detection bias”, “attrition bias”, “reporting bias”, and
134 “other bias”.²²

135

136 **3. Results**

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

146 *in vivo* studies, respectively. The shared references between *in vitro* and *in 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

171 adopted (Fig. 3). Most studies reported the use of a randomization process but failed to provide
172 details about the used methodology. Also, the details about the allocation concealment and
173 blinding were rather scarce or unclear in the author's opinion. Beyond these aspects, it was easy
174 for the reader to follow the flow of the animals throughout the study as no particularly numerous
175 experimental groups characterized the studies. The defined outcomes were very similar among the
176 studies and the failure in evaluating a specimen or the exclusion of an animal from the studies was
177 duly justified. Rigorous reporting of the data was not always respected and "problems that could
178 result in high risk of bias" were detected in the studies with few animals in which relevant
179 treatment effects might not be detected and false negative error (type II error) may be produced.
180 Thus, the retrieved *in vivo* studies about the percutaneous implant model for orthopedic
181 applications seem to be mostly affected by biases in randomization and blinding field of
182 application. These biases then affects many other items of this tool in which blinding plays a
183 central role.

184 3.3. Narrative results synthesis

185 3.3.1. *In vitro* studies. Detailed information about the study design and main outcomes of
186 included studies has been reported in ESI Table 1.† *In vitro* studies are wide-ranging and in some
187 specific cases very innovative.²³⁻⁴⁹ The cell types predominantly used are fibroblasts of human origin
188 in 12 studies²³⁻³⁴ and animal in 9 studies.³⁵⁻⁴³ Human keratinocytes were used in 10
189 studies^{24,26,28,31,35,44-48} and one study was performed using cells from animal origin.⁴⁹ Five studies were
190 performed analyzing both cell types,^{24,26,28,31,35} while the other studies were focused on fibroblasts or
191 keratinocytes. A 3D model was adopted in one study²³ and a co-culture with fibroblasts and
192 keratinocytes was used in another one.²⁶ In the remaining *in vitro* studies, the set-up was based on the
193 direct contact of a cell monolayer with materials or coating surfaces.

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

196 technique (MEW) was adopted by E. C. L. Bolle *et al.*, to print a poly(ϵ -caprolactone) porous
197 polymer structure in nano– micrometer range able to promote adhesion and colonization of
198 seeded fibroblasts, thus representing a suitable approach to modified the surface of percutaneous
199 devices.²³ Micro arc oxidation (MAO) is an electrochemical surface approach to generate a
200 microporous and adherent coating of TiO₂. The high potential of MAO approach is used to
201 produce discharges that penetrate the oxide coating allowing the doping with ions present in the
202 electrolytic solution as copper (Cu) or zinc (Zn) in order to confer also antibacterial properties to
203 the coating.^{36–39} The transfer of antibacterial properties to the coating has been studied not only
204 exploiting Cu and Zn which are relatively new in association with percutaneous devices, but also
205 by making coatings with nanostructured silver (Ag) content to inhibit biofilm formation on
206 percutaneous surfaces.^{32,33,46} MAO technique was also combined with the surface
207 functionalization with Fe₃O₄ magnetic nanoparticles to exploit the magnetic field gradient to
208 further enhance fibroblast cell response and to also explore the unknown bacterial killing effect
209 mediated by the production of reactive oxygen species by the Fe₃O₄ nanoparticles exposure.⁴⁰
210 MAO was also studied in combination with hydrothermal treatment (HT) to modify the
211 topography, obtaining features as nanoplates, nanorods and nanofibers, or the chemical
212 composition of the surfaces.

213 As expected, a great interest has been devoted to the nanostructure. In the present review, 13
214 studies are focused on the production of nanostructured features by adopting different
215 approaches as alkali heat treatment alone²⁵ or combined with HT,⁴¹ anodization^{26,31,50} electron
216 beam evaporation,^{45,48} microwave plasma chemical vapor.⁴⁷ The nanofeatures, generally
217 imposed onto metallic implants, improved the surface area thanks to a greater nanoscale
218 roughness. As a consequence, a greater availability of reactive sites to target the basic process of
219 protein adsorption, concentration and conformation is generated so determining an improvement in
220 cell adhesion, spreading and proliferation.

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

222 molecules. In these papers key molecules for skin tissue are investigated *i.e.*, transmembrane
223 proteins that mediate the interaction to the actin cytoskeleton as E-Cadherin,^{24,49} or extracellular
224 matrix proteins as laminin 332 or fibronectin (Fn)^{27,28,30} with an essential involvement in cell
225 motility and epithelial migration and organization. Fibroblast growth factor 2 (FGF-2) has been
226 studied in association with nanostructured surfaces and a significant improvement in keratinocyte
227 density after 5 days of culture was detected in comparison to conventional titanium⁴⁸ or fibroblast
228 control cultures.⁴² A. Trent *et al.*, explored the use of keratose or keratine extracted from human
229 hair fibers to realize a nano-keratin coating on titanium surfaces (as humans do not possess the
230 specific enzyme keratinases to degrade these proteins) to realize a resilient coating for
231 percutaneous device.²⁸ Indeed, fibronectin, a fundamental glycoprotein that mediates cell
232 interaction acting at different level of complexity, has been extensively investigated for the
233 percutaneous surface functionalization. Different chemical approaches to overcome the
234 desorption process that could occur during *in vivo* implantation have also been studied as
235 silanization of the surfaces or passivation.^{27,28,30}

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

240 **3.3.2. *In vivo* studies.** Thirty-two *in vivo* studies have been performed by using different
241 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
242 studies in rabbits,^{26,45,67,68} 3 studies in guinea pigs^{69–71} and 2 studies in swines/micro-pigs.^{72,73} One
243 study adopted a feline model⁷⁴ and another one reported the clinical and histological data
244 concerning 4 dogs which had undergone amputations due to tumors treated with direct skeletal
245 attachment osteointegrated system.⁷⁵ The present search provides a framework in which 3 type of
246 percutaneous implantation types have been adopted: (1) amputation of a limb and subsequent
247 implantation of the intramedullary, percutaneous and *exo*-prosthesis components in 6 studies; (2)

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

252 Three additional studies with a tibia percutaneous implant have been evaluated.^{52,54,68} Two studies
253 without the presence of the percutaneous component were also included in the review: one
254 study with the subcutaneous implant in the tibia of plates coated with adsorbed or silanized
255 fibronectin in comparison with control surfaces and coatings²⁷ and another study with the
256 implantation in the paraspinal muscles.⁵¹ The latter well investigated the influence of implant
257 porosity as the combination of different porosity dimension and strut size and the evaluation of
258 soft tissue penetration and colonization.⁵¹

259 In most of the evaluated papers, the devices were made of titanium (23 studies) and medical grade
260 Ti6Al4V alloy (14 studies), when specified. The evaluation of different combinations of porosities
261 and structures from the histological and histomorphometric point of views have been investigated
262 in different preclinical studies, as the optimal porosity range that could positively favour the
263 colonization of soft tissues and limit the downgrowth phenomenon has not yet been identified with
264 certainty. Some studies were focused on percutaneous implants coated with the commercial porous
265 titanium coating type “P2” or “K2” in comparison with smooth polished surfaces. These studies
266 well describe and elucidate the process of cell infiltration, neovascularization, epidermal
267 downgrowth and formation of a fibrous capsule induced by these types of coating already used to
268 coat the subdermal flange component of osteointegrated implants in clinical applications^{53,55,62,65,69–}
269 ^{71,76}

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

274 for the nanostructured surfaces in comparison to the polished titanium ones both in terms of
275 integration and reduction of epithelial downgrowth and of antibacterial activity.²⁶ Also *in vivo*, HA
276 coatings and surface functionalization with Fn, HAFn or HAFnAg, laminin 332, have been
277 evaluated as *in vivo* translation of promising results previously obtained with *in vitro*
278 studies.^{27,51,57,66} The investigation of antimicrobial agents appears to be less represented: E. L.
279 Perry *et al.*, evaluated the antimicrobial properties of CSA-13, a cationic steroid compound derived
280 from bile acids and loaded on polyurethane foam pads and placed in direct contact with the skin at the
281 exit site of a percutaneous tibial implant,⁵⁴ reporting it was not effective in preventing infections. H.
282 Qu *et al.*, experimented a sol-gel film to release triclosan (2,4,4'-trichloro-2-hydroxydiphenylether)
283 from a percutaneous tibial pin surface⁶⁸ and found no signs of infection around implants coated with
284 sol-gel film also detecting tissue normal healing and reduced epithelial downgrowth.

285 Finally, differently from the *in vitro* findings, polymers seem to be of considerable interest for
286 the percutaneous component. Polymers, natural or synthetic or a combination of both, represent
287 the most frequently biomaterials adopted for skin regeneration thanks to biocompatibility
288 properties and similarity to the skin (natural polymers) and good controllable mechanical and
289 physical properties (synthetic polymers).⁷⁶ The results and considerations reported in the
290 retrieved papers provide interesting insights that could help in developing better performing
291 materials or surface coatings. The research activities focused on naturally porous poly(2-
292 hydroxyethyl methacrylate) [poly(HEMA)], an hydrogel used for soft contact lens, evaluated
293 different pore size and interconnecting throat configuration, as it is or functionalized with an
294 adhesive agent (carbonyldiimidazole, CDI) or laminin332 or a combination of both. The performed
295 studies aimed to elucidate the skin regeneration and integration inside the polymer structure
296 from the morphological and compositional point of view^{56,57} as elasticity properties of this
297 device are close to those of the skin one, thus representing a possible candidate for percutaneous
298 coating. A. Oyane *et al.*, and K. Sasaki *et al.*, focused their investigation on ethylene-vinyl alcohol
299 copolymer (EVOH) coated with HA or with laminin⁴² or FGF-2⁶⁴ respectively immobilized into

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

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

313

314 **4. Discussion**

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

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

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

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

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

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

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

369 However, in the author's opinion several limitations exist that should be considered in the
370 experimental design of future studies about this topic regarding the *in vitro* studies. Bidimensional
371 (2D) cultures with one cell type certainly provide useful information on the possible cell behavior
372 but do not closely resemble the *in vivo* processes as 3D models in which structural, biochemical
373 and biomechanical interactions recreate more realistically the microenvironment complexity that
374 occur *in vivo*.⁸⁰ Cell–cell interaction as well as cell–matrix interactions in 3D models better reflect the *in*
375 *vivo* real condition as they prompt cells' commitment to differentiation developing different
376 functional conditions and subsets. This aspect is crucial for skin which is a tissue made up of

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

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

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

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

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

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

424 Flanges or subcutaneous barriers, adopted to increase the adhesion surface for fibroblasts and
425 keratinocytes, seem to provide a major stabilization of the soft tissue and to reduce the movement
426 of the epithelium at the interface thus reducing the downgrowth.^{55,74,75} The results obtained by S.
427 Jeyapalina *et al.*, with P2 porous-coated subdermal barrier seem to demonstrate a reduction in
428 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 could also 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 translatable 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

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

489

490 **5. Conclusion**

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

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

504

505 **Conflicts of interest**

506 There are no conflicts to declare.

507

508

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513 **References**

- 514 1 K. Ziegler-Graham, E. J. MacKenzie, P. L. Ephraim, T. G. Trivison and R. Brookmeyer,
515 Estimating the prevalence of limb loss in the United States: 2005 to 2050, *Arch. Phys. Med.*
516 *Rehabil.*, 2008, 89(3), 422–429, DOI: 10.1016/j.apmr.2007.11.005, PMID: 18295618.
- 517 2 P. Varma, M. G. Stineman and T. R. Dillingham, Epidemiology of limb loss, *Phys. Med. Rehabil.*
518 *Clin. North Am.*, 2014, 25(1), 1–8, DOI: 10.1016/j.pmr.2013.09.001, PMID: 24287235; PMCID:
519 PMC4533906.
- 520 3 K. Hagberg and R. Brånemark, Consequences of non-vascular trans-femoral amputation: a survey of
521 quality of life, prosthetic use and problems, *Prosthet. Orthot. Int.*, 2001, 25(3), 186–194, DOI:
522 10.1080/03093640108726601, PMID:11860092.
- 523 4 J. S. Hebert, M. Rehani and R. Stiegelmar, Osseointegration for Lower-Limb Amputation: A
524 Systematic Review of Clinical Outcomes, *JBJS Rev.*, 2017, 5(10), e10, DOI:
525 10.2106/JBJS.RVW.17.00037, PMID: 29087966.
- 526 5 R. Brånemark, P. I. Brånemark, B. Rydevik and R. R. Myers, Osseointegration in skeletal
527 reconstruction and rehabilitation: a review, *J. Rehabil. Res. Dev.*, 2001, 38(2), 175–181. PMID:
528 11392650.
- 529 6 K. Hagberg, R. Brånemark, B. Gunterberg and B. Rydevik, Osseointegrated trans-femoral
530 amputation prostheses: prospective results of general and condition-specific quality of life in 18
531 patients at 2-year follow-up, *Prosthet. Orthot. Int.*, 2008, 32(1), 29–41, DOI:
532 10.1080/03093640701553922, PMID: 18330803.
- 533 7 H. H. Aschoff, R. E. Kennon, J. M. Keggi and L. E. Rubin, Transcutaneous, distal femoral,
534 intramedullary attachment for above-the-knee prostheses: an endo-exo device, *J. Bone Jt. Surg.,*
535 *Am. Vol.*, 2010, 92(Suppl 2), 180–186, DOI: 10.2106/JBJS.J.00806, PMID: 21123601.
- 536 8 M. A. Muderis, K. Tetsworth, A. Khemka, S. Wilmot, B. Bosley, S. J. Lord and V. Glatt, The
537 Osseointegration Group of Australia Accelerated Protocol (OGAAP-1) for twostage osseointegrated

- 538 reconstruction of amputated limbs, *Bone Jt. J.*, 2016, 98-B(7), 952–960, DOI:
539 10.1302/0301620X.98B7.37547, PMID: 27365474.
- 540 9 Early Feasibility Study of the Percutaneous Osseointegrated Prosthesis (POP)—
541 <http://clinicaltrials.gov/ct2/show/study/NCT02720159>.
- 542 10 L. Guirao, B. Samitier, R. Tibau, J. Alós, M. Monago, M. Morales-Suarez-Varela and E.
543 Pleguezuelos, Distance and speed of walking in individuals with trans-femoral amputation fitted
544 with a distal weight-bearing implant, *Orthop. Traumatol. Surg. Res.*, 2018, 104(6), 929–933, DOI:
545 10.1016/j.otsr.2018.04.011, Epub 2018 Jun 1. PMID: 29864519.
- 546 11 R. L. McGough, M. A. Goodman, R. L. Randall, J. A. Forsberg, B. K. Potter and B.
547 Lindsey, The Compress® transcutaneous implant for rehabilitation following limb amputation,
548 *Unfallchirurg*, 2017, 120(4), 300–305, DOI: 10.1007/s00113-017-0339-9, English, PMID:
549 28275847.
- 550 12 Intraosseous transcutaneous amputation prosthesis-
551 <https://clinicaltrials.gov/ct2/show/NCT02491424?term=ITAP&rank=1>.
- 552 13 A. Thesleff, R. Brånemark, B. Håkansson and M. OrtizCatalan, Biomechanical Characterisation of
553 Bone-anchored Implant Systems for Amputation Limb Prostheses: A Systematic Review, *Ann.*
554 *Biomed. Eng.*, 2018, 46(3), 377–391, DOI: 10.1007/s10439-017-1976-4, Epub 2018 Jan 11. PMID:
555 29327257; PMCID: PMC5809556.
- 556 14 G. Liu, E. Chen, D. Lewis and G. Rao, Food and Drug Administration’s Humanitarian Device
557 Exemption Marketing Approval Pathway: Insights for Developing Devices for Rare Diseases, *J.*
558 *Med. Devices*, 2017, 11(3), 034701, DOI: 10.1115/1.4036333.
- 559 15 A. Peramo and C. L. Marcelo, Bioengineering the skin implant interface: the use of
560 regenerative therapies in implanted devices, *Ann. Biomed. Eng.*, 2010, 38(6), 2013–2031,
561 DOI: 10.1007/s10439-010-9937-1, Epub 2010 Feb 6. PMID: 20140520.
- 562 16 K. Affeld, J. Grosshauser, L. Goubergrits and U. Kertzscher, Percutaneous devices: a review of

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

565 17 J. Tillander, K. Hagberg, L. Hagberg and R. Brånemark, Osseointegrated titanium implants for
566 limb prostheses attachments: infectious complications, *Clin. Orthop. Relat. Res.*, 2010, 468(10),
567 2781–2788, DOI: 10.1007/s11999-010-1370-0, Epub 2010 May 15. PMID: 20473597; PMCID:
568 PMC2939339.

569 18 D. Wang, J. R. Gilbert, X. Zhang, B. Zhao, D. F. E. Ker and G. M. Cooper, Calvarial Versus Long
570 Bone: Implications for Tailoring Skeletal Tissue Engineering, *Tissue Eng., Part B*, 2020, 26(1),
571 46–63, DOI: 10.1089/ten.TEB.2018.0353, Epub 2019 Dec 18. PMID: 31588853.

572 19 M. J. Page, J. E. McKenzie, P. M. Bossuyt, I. Boutron, T. C. Hoffmann, C. D. Mulrow, L.
573 Shamseer, J. M. Tetzlaff, E. A. Akl, S. E. Brennan, R. Chou, J. Glanville, J. M.
574 Grimshaw, A. Hróbjartsson, M. M. Lalu, T. Li, E. W. Loder, E. Mayo-Wilson, S.
575 McDonald, L. A. McGuinness, L. A. Stewart, J. Thomas, A. C. Tricco, V. A. Welch, P.
576 Whiting and D. Moher, The PRISMA 2020 statement: an updated guideline for reporting
577 systematic reviews, *Br. Med. J.*, 2021, 372, n71, DOI: 10.1136/bmj.n71, PMID: 33782057;
578 PMCID: PMC8005924.

579 20 J. A. C. Sterne, J. Savović, M. J. Page, R. G. Elbers, S. Blencowe, I. Boutron, C. J.
580 Cates, H. Y. Cheng, M. S. Corbett, S. M. Eldridge, J. R. Emberson, M. A.
581 Hernán, S. Hopewell, A. Hróbjartsson, D. R. Junqueira, P. Jüni, J. J. Kirkham, T. Lasserson, T.
582 Li, A. McAleenan, B. C. Reeves, S. Shepperd, I. Shrier, L. A. Stewart, K. Tilling, I. R.
583 White, P. F. Whiting and J. P. T. Higgins, RoB 2: a revised tool for assessing risk of bias in
584 randomised trials, *Br. Med. J.*, 2019, 366, 14898, DOI: 10.1136/bmj.14898, PMID: 31462531.

585 21 A. Iliadi, D. Koletsi, S. N. Papageorgiou and T. Eliades, Safety Considerations for Thermoplastic-
586 Type Appliances Used as Orthodontic Aligners or Retainers. A Systematic Review and Meta-
587 Analysis of Clinical and In-Vitro Research, *Materials*, 2020, 13(8), 1843, DOI: 10.3390/
588 ma13081843, PMID: 32295303; PMCID: PMC7215465.

- 589 22 C. R. Hooijmans, M. M. Rovers, R. B. de Vries, M. Leenaars, M. Ritskes-Hoitinga and M. W.
590 Langendam, SYRCLE's risk of bias tool for animal studies, *BMC Med. Res. Methodol.*, 2014, 14,
591 43, DOI: 10.1186/1471-2288-14-43, PMID: 24667063; PMCID: PMC4230647.
- 592 23 E. C. L. Bolle, N. Bartnikowski, P. Haridas, T. J. Parker, J. F. Fraser, S. D. Gregory and T. R.
593 Dargaville, Improving skin integration around long-term percutaneous devices using fibrous
594 scaffolds in a reconstructed human skin equivalent model, *J. Biomed. Mater. Res., Part B*, 2020,
595 108(3), 738–749, DOI: 10.1002/jbm.b.34428, Epub 2019 Jun 6. PMID: 31169980.
- 596 24 J. Dehli, C. Karlsson, C. Bizelli-Silveira, X. Jiang, D. Kraft and M. Foss, E-cadherin mediated cell-
597 biomaterial interaction reduces migration of keratinocytes in-vitro, *Colloids Surf., B*, 2019, 180,
598 326–333, DOI: 10.1016/j.col-surf.2019.04.010, Epub 2019 Apr 5. PMID: 31075686.
- 599 25 M. Yamada, E. Kato, A. Yamamoto and K. Sakurai, A titanium surface with nano-ordered spikes
600 and pores enhances human dermal fibroblastic extracellular matrix production and integration of
601 collagen fibers, *Biomed. Mater.*, 2016, 11(1), 015010, DOI: 10.1088/1748-6041/11/1/015010,
602 PMID: 26835848.
- 603 26 J. Tan, C. Zhao, J. Zhou, K. Duan, J. Wang, X. Lu, J. Weng and B. Feng, Co-culturing
604 epidermal keratinocytes and dermal fibroblasts on nano-structured titanium surfaces, *Mater.*
605 *Sci. Eng., C*, 2017, 78, 288–295, DOI:10.1016/j.msec.2017.04.036, Epub 2017 Apr 7.
606 PMID:28575986.
- 607 27 M. Chimutengwende-Gordon, C. Pendegrass and G. Blunn, Enhancing the soft tissue seal
608 around intraosseous transcutaneous amputation prostheses using silanized fibronectin titanium
609 alloy, *Biomed. Mater.*, 2011, 6(2), 025008, DOI: 10.1088/1748-6041/6/2/025008, Epub 2011 Feb
610 28. PMID: 21358026.
- 611 28 A. Trent and M. E. Van Dyke, Development and characterization of a biomimetic coating for
612 percutaneous devices, *Colloids Surf., B*, 2019, 182, 110351, DOI:
613 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. Papat, 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. Sluszniaik, 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–

- 639 675, DOI: 10.1016/j.msec.2019.03.025, Epub 2019 Mar 12. PMID: 30948103.
- 640 36 L. Zhang, Y. Han and G. Tan, Hydroxyapatite nanorods patterned ZrO₂ bilayer coating on
641 zirconium for the application of percutaneous implants, *Colloids Surf., B*, 2015, 127, 8–14,
642 DOI: 10.1016/j.colsurfb.2015.01.017, Epub 2015 Jan 17. PMID: 25635615.
- 643 37 L. Zhang, J. Guo, X. Huang, Y. Zhang and Y. Han, The dual function of Cu-doped TiO₂ coatings
644 on titanium for application in percutaneous implants, *J. Mater. Chem. B*, 2016, 4(21), 3788–3800,
645 DOI: 10.1039/c6tb00563b, Epub 2016 May 6. PMID: 32263317.
- 646 38 L. Zhang, J. Zhang, F. Dai and Y. Han, Cytocompatibility and antibacterial activity of
647 nanostructured H₂Ti₅O₁₁·H₂O outlayered Zn-doped TiO₂ coatings on Ti for percutaneous
648 implants, *Sci. Rep.*, 2017, 7(1), 13951, DOI: 10.1038/s41598017-13954-4, PMID: 29066726;
649 PMCID: PMC5654996.
- 650 39 L. Zhang, J. Guo, T. Yan and Y. Han, Fibroblast responses and antibacterial activity of Cu and Zn
651 co-doped TiO₂ for percutaneous implants, *Appl. Surf. Sci.*, 2018, 434, 633–642, DOI:
652 10.1016/j.apsusc.2017.10.169.
- 653 40 K. Li, S. Liu, Y. Xue, L. Zhang and Y. Han, A superparamagnetic Fe₃O₄-TiO₂ composite coating
654 on titanium by micro-arc oxidation for percutaneous implants, *J. Mater. Chem. B*, 2019, 7(34),
655 5265–5276, DOI: 10.1039/c9tb01096c, Epub 2019 Aug 6. PMID: 31384861.
- 656 41 K. Li, Y. Xue, T. Yan, L. Zhang and Y. Han, Si substituted hydroxyapatite nanorods on Ti for
657 percutaneous implants, *Bioact. Mater.*, 2020, 5(1), 116–123, DOI:
658 10.1016/j.bioactmat.2020.01.001, PMID: 32021946; PMCID: PMC6994265.
- 659 42 K. Sasaki, A. Oyane, K. Hyodo, A. Ito, Y. Sogo, M. Kamitakahara and K. Ioku,
660 Preparation and biological evaluation of a fibroblast growth factor-2-apatite composite layer on
661 polymeric material, *Biomed. Mater.*, 2010, 5(6), 065008, DOI: 10.1088/1748-6041/5/6/065008,
662 Epub 2010 Oct 22. PMID: 20966534.
- 663 43 M. A. Shevtsov, O. V. Galibin, N. M. Yuditceva, 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.

714 56 P. Fleckman, M. Usui, G. Zhao, R. Underwood, M. Maginness, A.
715 Marshall, C. Glaister, B. Ratner and J. Olerud, Cutaneous and inflammatory response to
716 longterm percutaneous implants of sphere-templated porous/ solid poly(HEMA) and silicone in
717 mice, *J. Biomed. Mater. Res., Part A*, 2012, 100(5), 1256–1268, DOI: 10.1002/jbm.a.34012, Epub
718 2012 Feb 23. PMID: 22359383; PMCID: PMC3506026.

719 57 Y. Fukano, M. L. Usui, R. A. Underwood, S. Isenhath, A. J. Marshall, K. D. Hauch, B. D.
720 Ratner, J. E. Olerud and Fleckman, Epidermal and dermal integration into sphere-templated
721 porous poly(2-hydroxyethyl methacrylate) implants in mice, *J. Biomed. Mater. Res., Part A*, 2010,
722 94(4), 1172–1186, DOI: 10.1002/jbm.a.32798, PMID: 20694984; PMCID: PMC2998188.

723 58 R. A. Underwood, M. L. Usui, G. Zhao, K. D. Hauch, M. M. Takeno, B. D. Ratner, A.
724 J. Marshall, X. Shi, J. E. Olerud and P. Fleckman, Quantifying the effect of pore size and surface
725 treatment on epidermal incorporation into percutaneously implanted sphere-templated porous
726 biomaterials in mice, *J. Biomed. Mater. Res., Part A*, 2011, 98(4), 499–508, DOI:
727 10.1002/jbm.a.33125, Epub 2011 Jun 16. PMID: 21681942; PMCID: PMC4684638.

728 59 P. Pholpabu, S. S. Yerneni, C. Zhu, P. G. Campbell and C. J. Bettinger, Controlled Release of
729 Small Molecules from Elastomers for Reducing Epidermal Downgrowth in Percutaneous Devices,
730 *ACS Biomater. Sci. Eng.*, 2016, 2(9), 1464–1470, DOI: 10.1021/acsbiomaterials.6b00192, Epub
731 2016 Aug 18. PMID: 33440584.

732 60 T. Calliess, I. Bartsch, M. Haupt, M. Reebmann, M. Schwarze, M. Stiesch, C.
733 Pfaffenroth, M. Sluszniak, W. Dempwolf, H. Menzel, F. Witte and E. Willbold, In vivo
734 comparative study of tissue reaction to bare and antimicrobial polymer coated transcutaneous
735 implants, *Mater. Sci. Eng., C*, 2016, 61, 712–719, DOI: 10.1016/j.msec.2015.12.095,
736 Epub 2016 Jan 4. PMID: 26838901.

737 61 B. J. Farrell, B. I. Prilutsky, J. M. Ritter, S. Kelley, K. Popat and M. Pitkin, Effects of pore size,
738 implantation time, and nano-surface properties on rat skin ingrowth into percutaneous porous

739 titanium implants, *J. Biomed. Mater. Res., Part A*, 2014, 102(5), 1305–1315, DOI:
740 10.1002/jbm.a.34807, Epub 2013 Jun 7. PMID: 23703928; PMCID: PMC3871976.

741 62 D. Isackson, K. J. Cook, L. D. McGill and K. N. Bachus, Mesenchymal stem cells increase
742 collagen infiltration and improve wound healing response to porous titanium percutaneous
743 implants, *Med. Eng. Phys.*, 2013, 35(6), 743–753, DOI: 10.1016/j.medengphy.2012.08.002, Epub
744 2012 Aug 31. PMID: 22940446; PMCID: PMC3529992.

745 63 K. Nam, R. Matsushima, T. Kimura, T. Fujisato and A. Kishida, In vivo characterization of
746 a decellularized dermis-polymer complex for use in percutaneous devices, *Artif. Organs*, 2014,
747 38(12), 1060–1065, DOI: 10.1111/aor.12330, Epub 2014 Jun 25. PMID: 24962020.

748 64 A. Oyane, K. Hyodo, M. Uchida, Y. Sogo and A. Ito, Preliminary in vivo study of apatite and
749 laminin-apatite composite layers on polymeric percutaneous implants, *J. Biomed. Mater. Res.,*
750 *Part B*, 2011, 97(1), 96–104, DOI: 10.1002/jbm.b.31790, Epub 2011 Feb 2. PMID: 21290587.

751 65 D. R. L. Pawar, S. Jeyapalina, K. Hafer and K. N. Bachus, Influence of negative pressure wound
752 therapy on peri-prosthetic tissue vascularization and inflammation around porous titanium
753 percutaneous devices, *J. Biomed. Mater. Res., Part B*, 2019, 107(6), 2091–2101 DOI:
754 10.1002/jbm.b.34302, Epub 2019 Jan 10. PMID: 30629801.

755 66 S. Asoda, T. Arita and K. Takakuda, Mechanical attachment of soft tissue to dental and
756 maxillofacial implants with mesh structures: an experiment in percutaneous model, *J.*
757 *Biomed. Mater. Res., Part B*, 2013, 101(4), 553–559, DOI: 10.1002/jbm.b.32855, Epub 2012 Dec
758 20. PMID: 23281217.

759 67 D. Isackson, L. D. McGill and K. N. Bachus, Percutaneous implants with porous titanium dermal
760 barriers: an in vivo evaluation of infection risk, *Med. Eng. Phys.*, 2011, 33(4), 418–426, DOI:
761 10.1016/j.medengphy.2010.11.007, Epub 2010 Dec 10. PMID: 21145778; PMCID: PMC3071885.

762 68 H. Qu, C. Knabe, S. Radin, J. Garino and P. Ducheyne, Percutaneous external fixator pins with
763 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

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

816 84 S. Yerneni, Y. Dhaher and T. A. Kuiken, A computational model for stress reduction at the skin-
817 implant interface of osseointegrated prostheses, *J. Biomed. Mater. Res., Part A*, 2012, 100(4), 911–
818 917, DOI: 10.1002/jbm.a.34028, Epub 2012 Jan 24. PMID: 22275149.

819 85 J. A. S. Quaresma, Organization of the Skin Immune System and Compartmentalized Immune
820 Responses in Infectious Diseases, *Clin. Microbiol. Rev.*, 2019, 32(4), e00034–e00018, DOI:
821 10.1128/CMR.00034-18, PMID: 31366611; PMCID: PMC6750136.

822 86 A. V. Nguyen and A. M. Soulika, The Dynamics of the Skin’s Immune System, *Int. J. Mol. Sci.*,
823 2019, 20(8), 1811, DOI: 10.3390/ijms20081811, PMID: 31013709; PMCID: PMC6515324.

824 87 M. Coates, S. Blanchard and A. S. MacLeod, Innate antimicrobial immunity in the skin: A
825 protective barrier against bacteria, viruses, and fungi, *PLoS Pathog.*, 2018, 14(12), e1007353, DOI:
826 10.1371/journal.ppat.1007353, PMID: 30522130; PMCID: PMC6283644.

827 88 T. G. A. Calon, M. Trobos, M. L. Johansson, J. van Tongeren, M. van der Lugt-Degen, A.
828 M. L. Janssen, P. H. M. Savelkoul, R. J. Stokroos and A. E. Budding, Microbiome on the Bone-
829 Anchored Hearing System: A Prospective Study, *Front. Microbiol.*, 2019, 10, 799, DOI:
830 10.3389/fmicb.2019.00799, PMID: 31105654; PMCID: PMC6498861.

831 89 T. G. A. Calon, J. van Tongeren, O. Omar, M. L. Johansson and R. J. Stokroos, Cytokine
832 expression profile in the boneanchored hearing system: 12-week results from a prospective
833 randomized, controlled study, *Clin. Implant. Dent. Relat. Res.*, 2018, 20(4), 606–616, DOI:
834 10.1111/cid.12615, Epub 2018 Apr 27. PMID: 29701288; PMCID: PMC6099213.

835 90 J. P. Beck, M. Grogan, B. T. Bennett, S. Jeyapalina, J. Agarwal, C. Bartow-McKenney, J.
836 Bugayev, E. Kubiak, S. Sinclair and E. Grice, Analysis of the Stomal Microbiota of a Percutaneous
837 Osseointegrated Prosthesis: A Longitudinal Prospective Cohort Study, *J. Orthop. Res.*, 2019,
838 37(12), 2645–2654, DOI: 10.1002/jor.24421, Epub 2019 Aug 2. PMID: 31317568.

839 91 <https://www.osseointegration.eu/stoma-care>.

840 92 H. D. Zomer and A. G. Trentin, Skin wound healing in humans and mice: Challenges in
841 translational research, *J. Dermatol. Sci.*, 2018, 90(1), 3–12, DOI:
842 10.1016/j.jdermsci.2017.12.009, Epub 2017 Dec 26. PMID: 29289417.

843 93 N. Naldaiz-Gastesi, O. A. Bahri, A. López de Munain, K. J. A. McCullagh and A. Izeta, The
844 panniculus carnosus muscle: an evolutionary enigma at the intersection of distinct research fields,
845 *J. Anat.*, 2018, 233(3), 275–288, DOI: 10.1111/joa.12840, Epub ahead of print. PMID: 29893024;
846 PMCID: PMC6081499.

847 94 L. Rittié, Cellular mechanisms of skin repair in humans and other mammals, *J. Cell Commun.*
848 *Signal.*, 2016, 10(2), 103–120, DOI: 10.1007/s12079-016-0330-1, Epub 2016 May 12. PMID:
849 27170326; PMCID: PMC4882309.

850 95 A. Grada, J. Mervis and V. Falanga, Research Techniques Made Simple: Animal Models of Wound
851 Healing, *J. Invest. Dermatol.*, 2018, 138(10), 2095–2105, DOI: 10.1016/j.jid.2018.08.005,
852 PMID: 30244718.

853 96 M. Al Muderis, A. Khemka, S. J. Lord, H. Van de Meent and J. P. M. Frölke, Safety of
854 Osseointegrated Implants for Transfemoral Amputees: A Two-Center Prospective Cohort Study,
855 *The Journal of Bone and Joint Surgery, American Volume*, 2016, 98(11), 900–909, DOI: 10.2106/
856 JBJS.15.00808.