**Supplementary Table 2.** Summary of the *in vivo* studies included in the systematic review

| EXPERIMENTAL DESIGN | MATERIALS/COATINGS | PERFORMED EVALUATIONS | MAIN OUTCOME | MAIN CONCLUSION | REF |
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| *SOFT TISSUE IMPLANTS* | | | | | |
| Three sheep submitted to subcutaneous implant of materials<br><br>4 weeks | Ti alloy plates coated with:<br><br>- Adsorbed fibronectin (AdFn)<br>- Silanized Fibronectin (SiFn)<br><br>*Vs* control surfaces:<br><br>- Polished Ti alloy (Pol),<br>- silanized only (Si),<br>- hydroxyapatite (HA)<br>- HA with fibronectin (HAFn). | Histology | No significant differences among HA, HAFn and SiFn<br><br>↑ cell alignment on HA, HAFn and SiFn *vs* Pol and AdFn<br><br>↑ soft-tissue attachment on HAFn ≥ HA ≥ SiFn.<br><br>↓ soft-tissue attachment on Pol, Si and AdFn | HAFn and SiFn increased soft-tissue attachment and produced better cell alignment than other surfaces. | Chimutengwende-Gordon, 2011* |
| Six sheep submitted to intramuscular material implantation<br><br>4 weeks | Ti6V4Al implants realized with Electron Beam Manufacturing with different porosity and strut size<br><br>Nine groups (pore size/strut size um$^2$):<br><br>Group 1: 1000/400<br><br>Group 2: 1000/200<br><br>Group 3: 700/400<br><br>Group 4: 700/300 | Histology<br><br>Histomorphometry on outer edges (zones 1 and 2) and central region (zone 3) | Group 1, 2 and 4 showed the highest % of device filling with dense, well-ordered soft tissues<br><br>↑ cell nuclei density in Groups 1, 2 and 4 *vs* all other groups.<br><br>↑ cell number in Group 2 *vs* all other groups.<br><br>↑ re-vascularization in Groups 2 and 4 *vs* all other groups in zone | Groups 1, 2 and 4 exhibited better performances in terms of tissue infiltration and revascularization across the entire structure *vs* the other Groups | Chimutengwende-Gordon, 2018 |

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| | Group 5: 700/200<br><br>Group 6: 500/400<br><br>Group 7: 500/300<br><br>Group 8: 500/200<br><br>Group 9: 200/300 | | 3.<br><br>Re-vascularization was not observed throughout implants in Groups 5, 6 and 7 | | |
| | | | **PERCUTANEOUS IMPLANTS** | | |
| Six sheep submitted to bone pin and subcutaneous flange implantation at the tibial level<br><br>4 weeks | Pins realized with laser-sintered porous Ti alloy flange (pore size 700 μm and strut size 300 μm; porosity 18%).<br><br>The flanges were:<br><br>- uncoated (PT);<br>Coated with:<br><br>- electrochemically deposited HA (PT-HA);<br>- HA with fibronectin (PT-HAFn);<br>- HA with silver (PT-HAAg);<br>- HA with silver and fibronectin (PT-HAAgFn).<br><br>*VS* controls:<br><br>Flat flange design (DF) with drilled holes (700 μm diameter) and a<br><br>tapered intraosseous stem | Histology | ↑ epidermal downgrowth in SP *vs* DF.<br><br>↓ epithelial downgrowth in PT, PT-HAAg, PT-HAAgFn and PTHAFn *vs* DF.<br><br>↓ epithelial attachment in SP<br><br>↑ epithelial attachment for DF and no difference between DF and PT implants.<br><br>↑ dermal attachment for DF *vs* SP. Improved attachment in PT-HAFn *vs* PT-HA and in PT-HAAgFn *vs* PT-HAAg.<br><br>↑ soft-tissue integration in PT *vs* DF. | A porous Ti flange with interconnected<br><br>pores improved soft-tissue integration.<br><br>It reduces epithelial<br><br>downgrowth and increases dermal attachment, cell nuclei density and<br><br>blood vessel ingrowth.<br><br>The coatings did not show any statistically significant advantages over PT design. | Chimutengwende-Gordon, 2017 |

| | | | The addition of coatings did not result in any increases in soft-tissue fill %<br><br>↑ cell nuclei and blood vessel within the inner pores of the PT flange *vs* the DF.<br><br>Coatings did not result in further increases in blood vessel ingrowth. | | |
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| | The flange and the stem were coated with plasma sprayed HA.<br><br>An uncoated straight pin (SP) without a flange was included as a control for the DF design. | | | | |
| Twenty-three sheep submitted to a single -stage amputation and implantation surgery<br><br>Animals divided into two groups:<br><br>Group 1: Experimental group (porous-coated implant);<br><br>Group 2: Control group (smooth implant).<br><br>9 months | Ti-6Al-4V intramedullary component textured by grit blasting; subdermal barrier coated with pure Ti porous coating, P2 with surface roughness (Ra)= 113 ± 25 mm and porosity = 52 ± 12% in Group 1; polished smooth in Group 2<br><br>In both groups a porous-coated structure P2 type coating at the base of the endo-prosthetic part was present. | Microbiological investigation<br><br>Histology | Group 2: one postoperative fracture and one severe infection; Two superficial infection<br><br>hyper-cellular skin–implant interfaces with fibroblasts and lymphocytes.<br><br>↑ degrees of proximal skin migration *vs* G1 group<br><br>Group 1: Skin–implant interfaces: normal skin flora and/or environmental organisms in almost all implants. | No superficial or deep periprosthetic infection in Group 1 group<br><br>Greater marsupialization in Group 2 group *vs* Group 1 Group | Jeyapalina, 2012 |
| Eight sheep submitted to a single-stage amputation and | Ti-6Al-4V intramedullary component textured by grit | Histology and | 2/8 animals were removed from | The porous subdermal coating seems to be | Jeyapalina, 2017 |

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| implantation surgery<br><br>2 years | blasting; the subdermal component, the collar and the most distal part coated with a thick 500-750 um pure Ti porous coating, P2 (porosity = 52 ± 12%). | immunohistochemistry | the study<br><br>1/6 animal infected at 15-months.<br><br>The sub-dermal porous-coating was exposed in all animals<br><br>Fibrous soft-tissue ingrowth in porous coating in all animals.<br><br>In 3/5 a transient thin epithelial attachment.<br><br>The average rate of downgrowth was 0.343 ± 0.07 mm/month.<br><br>↑ degree of inflammatory cells and marker of migrating keratinocytes in sample with no epithelial attachment | able to protect from infection development in short term.<br><br>No evidence of permanent sealed skin/implant attachment and of skin wound healing | |
| Twenty sheep submitted to a percutaneous implant with a medial end protruding 1.5–3 cm outside the skin for the attachment of polyurethane foam pad for the delivery of an antibacterial agent | Machined Ti alloy passivated using nitric acid | Microbiology<br><br>Histology | 9/10 in Group 1 and 8/10 in Group 2 had bacteria<br><br>in blood, bone, and/or soft tissue. | CSA-13 did not prevent pin track infection as 95% (19 out of 20) of the animals were infected. | Perry, 2010 |

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| (cationic steroid antimicrobial CSA-13)<br><br>Group 1: antibacterial agent (CSA-13)<br><br>Group 2: no antibacterial agent<br><br>6 months | | SEM | 17/20 of the sheep had positive histology results for Gram-positive in both Groups<br><br>↑ clinical implant loosening in Group 1 (9/10) *vs* Group 2 (2/10).<br><br>↓ osteointegration and bone healing in Group 1 *vs* Group 2 | | |
| Eighty-six sheep submitted to a single-stage amputation and implantation surgery.<br><br>Nine animals were implanted with control surfaces, and the remainder with porous-coated surfaces.<br><br>3, 6, 9 and 12-months | Ti6Al4V customized implants.<br><br>Implants included three major design components: (i) Mores taper; (ii) sub-dermal fixation surface; and (iii) intramedullary portion.<br><br>In the control group sub-dermal surfaces were machined and smooth polished<br><br>In the experimental group the subdermal component was coated with commercially pure Ti | Histological evaluation of the degree of epithelial integration<br><br>Measure of epithelial regression and sub-epithelial/implant adhesion | The mean rate of cutaneous regression was 0.90 ±0.23 mm/months at 3 months, 0.56 ± 0.15 at 9 months and 0.44 ±0.22 at 12 months in the experimental group with a decrease in the regression.<br><br>↑ skin marsupialization in the control group at 9 months<br><br>At 3-months in the experimental group the 86.1% of the subdermal surfaces were covered by sub-epithelial soft-tissue while at 12 months the percentage decreased up to 70.9%. | Porous sub-dermal surface at the skin/implant interface can limit skin regression in the presence of relative motion | Holt, 2013a |

| | porous coating (P2 type) while the intramedullary portion of the implant was grit blasted | | | | |
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| Eight mice submitted to soft tissue pillar implants with a protruding end of 4 mm and sealed with dental bonding agent<br><br>4 weeks | Ti pillars treated with MAO technique to obtain a surface functionalization with Magnetic $Fe_3O_4$ nanoparticles (NPs) at 0 (CA) and 2.25 g L-1 (FT3) concentration<br><br>vs polished Ti (pol Ti). | Histology | Pol Ti: migration of the skin along the implant with little skin adhesion on the surface.<br><br>CA: ↓ downgrowth and ↑ inflammatory response vs pol Ti<br><br>FT3: little skin migration along the implant and no fibrous tissue formation. Weak inflammatory response vs CA. | Superparamagnetic $TiO_2$ coating with Fe prevented the soft tissue recession and the inflammatory reaction representing a potential coating to be applied on the percutaneous implant. | Li, 2019* |
| Eight mice in which a through-and-through fashion, approach was used to create two wound (exit) sites in which the pillars were implanted with the two ends of the pilar extending from the wound sites<br><br>4 weeks | Ti pillars treated with alkali-heat treatment (AHT) followed with hydrothermal treatment (HT) to obtain a HA nanorod and Si-HA nanorod with the addition of Si<br><br>vs polished Ti (pol Ti) | Histology | Pol Ti: ↓ skin adhesion and higher skin migration and fibrous capsule (about 400 um) on implant surface<br><br>HA: ↓ skin downgrowth and fibrous capsule (100um) vs Pol Ti.<br><br>Si-HA: ↓ skin migration and no fibrous capsule | Pol Ti: weak integration<br><br>Si-HA: tight seal between the underlying dermis and the implant surface | Li, 2020* |
| Thirty-two mice.<br><br>A needle was used to pierce the skin in a through-and-through fashion, creating two | A porous/solid poly(HEMA) with pores measuring 36 um in diameter and interconnecting throats | Measurements of the contraction of the skin that "bridges" over the implant | ↑ contraction of the upper bridge region of the skin for silicone implants vs poly(HEMA) implants at each time | No marsupialization, foreign body encapsulation or infection were observed in long-term | Fleckman, 2012 |

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| wound (exit) sites 0.5 cm apart midline between the scapulae and 1 cm posterior to the ears.<br><br>The porous rod was inserted leaving the rod implanted through the skin with the two ends extending from two exit sites.<br><br>Each mouse was implanted with 1 porous/solid poly(2-hydroxyethyl methacrylate) [poly(HEMA)] rod and 1 porous/solid silicone rod.<br><br>14 days, 1, 3 and 6 months | measuring 14 um.<br><br>As control material, porous/solid silicon material with the same porosities | Histology<br><br>Immunohistochemistry | Poly(HEMA)<br><br>At 14 day: 0 broken at the exit site; 0 skin covering<br><br>1 month: 0 broken at the exit site; 0 skin covering<br><br>3 months: 4/10 broken at the exit site; 1/10 skin covering<br><br>6 months: 5/14 broken at the exit site; 8/14 skin covering<br><br>Keratinocytes penetration into the pores of Poly(HEMA) from both the ventral and dorsal regions of the epidermis, with formation of a sheath in the dorsal region. Endothelial cells at all-time points. Vessel formation inside the voids and progressive collagen I maturation during experimental times. Macrophages identified within pores throughout the implants at all-time points<br><br>Silicon<br><br>At 14 day: 0 broken at the exit site; 0 skin covering<br><br>1 month: 0 broken at the exit | implantation of sphere-templated porous poly(HEMA) and silicone | |

| | | | site; 0 skin covering | | |
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| | | | 3 months: 0 broken at the exit site; 0 skin covering | | |
| | | | 6 months: 0 broken at the exit site; 2/14 skin covering | | |
| | | | Air-exposed regions of the implants broke off in most samples during sectioning. In the available samples | | |
| | | | epidermis implants appeared to | | |
| | | | respond similarly to the poly(HEMA) implants. Keratinocytes showed limited migration at all-time points. Vessels were seen throughout all time points. | | |
| | | | Monocytes/macrophages was similar to that of poly(HEMA) | | |
| Seventy-nine mice<br><br>A needle was used to pierce the skin in a through-and-through fashion, creating two wound (exit) sites 0.5 cm apart midline between the scapulae and 1 cm posterior to the ears.<br><br>7, 14, and 28 days | Cross- linked sphere-templated poly(HEMA). (A)poly(2-hydroxyethyl methacrylate) [poly(HEMA)] with uniform 40 um pores and 16 um pore interconnects (throats).<br><br>Different surface treatments:<br><br>(1) untreated and stored in | Histology<br><br>Immunohistochemistry<br><br>Electron microscopy (TEM) to qualitatively evaluate the integration implant/skin | 24/24 intact implant at 7 days, 63/64 intact at 14 days, and 57/70 intact at 28 days.<br><br>No signs of infection at the implant/skin interface for all implants.<br><br>Crust-like structures at exit sites in all implants at 7 and 14 days and slight downgrowth of the | Sphere-templated polymers with 40 um pores was successful with all surface treatment in stimulating dermal and epidermal layers integration | Fukano, 2010 |

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| | PBS;<br><br>(2) surface treated with carbonyldiimidazole (CDI);<br><br>(3) surface modified with CDI and reacted with partially purified human laminin 332 (CDI/lam 332), | | epidermis along the implant.<br><br>All immunohistochemical markers were detected within the pores at all time points and surface treatments.<br><br>Blood vessels formation and collagen bundles at 14 and 28 day in all implants. | | |
| Thirty-six mice in which a through-and-through fashion, approach was used to create two wound (exit) sites in which the rods were implanted with the two ends of the rod extending from the two wound sites<br><br>14 days | Porous poly(HEMA) cylindrical rods with 20, 40 or 60 µm pores and inter-connecting throats that are 40% of the pore size.<br><br>Rods were used untreated or surface modified with with carbonyldiimidazole, as adhesive agent (CDI) | Histology<br><br>Immunohistochemistry<br><br>Migrating front measurements | A tight contact between epidermis and materials in all samples.<br><br>↑ average migration distance and migrating front distance for the 40 and 60 µm *vs* 20µm porous materials<br><br>Migration was unaffected by surface modification (without considering of pore size and healing duration as factors)<br><br>Epithelial integration after 3 days | Epidermal growth into porous biomaterial was enhanced using pore sizes greater than 20µm and is minimally influenced by surface treatment | Underwood, 2011 |
| Mice in which a through-and-through fashion approach | A biodegradable elastomer cylinder of poly | Histology | Comparable density of macrophages at 3 days for blank | CLA-eluting PGS-CinA elastomers represent | Pholpabu, 2016 |

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| was used to create two wound (exit) sites in which the cylinders were implanted with the two ends of the rod extending from the two wound sites<br><br>3 ,7, 10 and 14 days | (glycerol-*co*-sebacate)-cinnamate (PGS-CinA), loaded with (lipopolysaccharide - LPS), conjugated linoleic acid (CLA) or a combination LPS+CLA *vs* the blank polymer | Immunohistochemistry | and CLA-eluting PGS-CinA.<br><br>↑ macrophages density in CLA-eluting material *vs* blank at 7days<br><br>CLA-eluting material and the blank PGS-CinA material reduce epidermal downgrowth at 14 d *vs* LPS-PGS-CinA<br><br>↓ epidermal downgrowth at 14 days for CLA eluting material and the blank vs LPS-PGS-CinA<br><br>CLA-eluting material best<br><br>preserve the bridge length at 7 d *vs* other materials | a viable coating strategy to reduce epidermal downgrowth in percutaneous devices. | |
| Eighty-two mice implanted on the dorsal skin with a protruding pin<br><br>7, 14 and 21 and 168 days | Polished Ti alloy (Ti6Al4V) implant with a perforated round disc with eight peripheral holes for the subcutaneous implant and a central pin as transcutaneous<br><br>part of the implant.<br><br>Half of these polished implants were used as controls while the other half | Histology<br><br>Immunohistochemistry | No differences between control and coated implants after 7 and 14 days for scar formation.<br><br>At 21 days, the control showed a thicker scar tissue *vs* the VP-co-DMMEP-coated implants.<br><br>No differences in terms of degree of epithelialization. | The results suggested that the antimicrobial effect appeared to be temporary. | Calliess, 2016 |

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| | were dip coated with polymers (copolymer dimethyl (2-methacryloyloxy-ethyl) phosphonate and 4-vinylpyridine - VP-co-DMMEP at a ratio of 30:70) with antimicrobial properties | | After 7 days, both groups showed an average downgrowth of about 400 μm, which increased to about twofold at 14 days. At 21 days the values decreased in both groups.<br><br>No significant differences regarding the frequency of inflammations at the skin-implant interface.<br><br>Infective events occurred between days 50 and 75. In control group 6/12 and 5/10 of the polymer group reached the endpoint of 168 days.<br><br>In the control group 7 animals showed infection all around the implants and deeper tissue regions.<br><br>In the VP-co- DMMEP group, 2 animals showed infection in deeper tissue regions. | | |
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| Eighteen rats submitted to films percutaneous implant | Ethylene–vinyl alcohol copolymer (EVOH) films also coated with a liquid- | Histology | ↑ downgrowth and pocket depth for uncoated film *vs* F0 and F4 | Good biocompatibility for the HA coating supplemented with | Sasaki, 2010* |

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| protruding 3 mm<br><br>14 days | phase coating process with a solution of HA supplemented with 0 or 4, µg mL−1 of Fibroblast Growth factor – 2 (FGF) (F0, F4) | | No difference between F0 and F4 in downgrowth and pocket depth.<br><br>5/6 sample in uncoated group has a fibrous connective tissue with a thickness higher than 200 µm (83% of incidence).<br><br>1/6 (17%) sample in F0 and F4 has a fibrous connective tissue with a thickness higher than 200 µm, while the incidence of fibrous connective tissue with a thickness lower than 50 um was 3/6 (50%) in F0 and 2/6 (33%) in F4. | FGF and retaining of FGF2activity in vivo | |
| Twenty five hairless rats divided into 3.<br><br>1) Small, implanted with small pore rod;<br>2) Large, implanted with larger pore rod;<br>3) Nano, implanted with small pore rod with nanotubular surface treatment<br><br>3 or 6 weeks.<br><br>Sprague Dawley rats divided | Porous Ti rods (porosity 45±5%) sintered with Ti6AL4V powders with two different porosities:<br><br>Small, 40–100 µm, and Large, 100–160 µm with and without nanotubular surface (surfaces pore diameters ranging from 50 - 250 nm) obtained with anodization<br><br>Solid Ti implants were used for comparison. | Histology of the skin/implant interface<br><br>Skin ingrowth area and fraction of filling of implant pores | 1/5 solid implant remained up to 3 weeks (4/5 were extruded by skin or removed by the rat).<br><br>1/35 porous implant was removed due to clinical signs of infection.<br><br>Several porous implants were removed because the extruded length reached the settled humane endpoint. | All types of porous Ti implants demonstrated skin tissue ingrowth with and without nanotubular surface | Farrell, 2014b |

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| into small and nano groups<br><br>6 weeks.<br><br>Five Sprague Dawley rats implanted with solid titanium implants as a control group.<br><br>Rods were implanted into the skin between the scapulae leaving 3–5 mm of implant exposed | | | Signs of tissue ingrowth (fibrovascular tissue) into the pores of all porous implant.<br><br>Diffuse fibrovascular ingrowth into the pores of nanotubular surface (5/5)<br><br>↑ area filled with skin for implant at 4–6 weeks (50%) *vs* 3 weeks (30%).<br><br>No significant difference in skin ingrowth among implant type. | | |
| Twenty-five rats receiving each two percutaneous implants onto subcutaneous pocket created in the dorsum and with a post protruding 5 mm:<br><br>1) one implant treated with 6 ×10$^6$ Mesenchymal Stromal Cells (MSC) obtained from syngeneic donors;<br>2) one untreated (control).<br><br>0, 3 7, 28 and 56 days | Percutaneous implant of a Ti6Al4V substrate. The implant was surfaced with pure Ti porous coating (P2 type) with  a ~55% porosity and an average pore size of ~360 um | Histology and measurement of several parameters (cellular infiltration, neovascularization, tissue ingrowth, epidermal downgrowth, and fibrous encapsulation) | Day 0: 3/3 treated implants and 1/3 of the untreated filled with macrophages and red blood cells<br><br>Day 3: treated implants ↑collagen matrix infiltration and ↓ fibrin/serum presence *vs* untreated; ↑ macrophages and lymphocytes in treated and ↑ polymorphonucleated cells in untreated.<br><br>Day 7: Fibrin clot resolved in both type of implants; Integration | MSC can influence the wound healing promoting the resolution of the inflammatory phase in percutaneous implant | Isackson, 2013 |

| | | | of the epidermidis and granulomatous inflammation. Minimal vascular formation in both type of implants | | |
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| Rats in which the device was implanted in subcutaneous pockets leaving the post to protrude through the skin<br><br>4, 8 and 12 weeks | Device with a subdermal component in porcine decellularized dermis partially embedded in poly(methyl methacrylate - PMMA), with a percutaneous polymer rod in PMMA | Histology | Detection of macrophages at 4 weeks<br><br>No foreign body giant cells at 8 weeks<br><br>Blood vessel formation inside a collagenous tissue layer without capsule formation.<br><br>No signs of swelling, bleeding, clot formation, or keloid formation in the exit site at 12 weeks. | The decellularized tissue showed high biocompatibility with no signs of downgrowth of the epidermis | Nam, 2014 |
| Rat implantation in a percutaneously fashion created in the scalp leaving the specimen protruding 3 mm from the skin<br><br>14 days | Ethylene-vinyl alcohol copolymer (EV) film (50-60 um) coated with HA (EVCP) or laminin-HA (EVLCP) | Histology<br><br>Histomorphometry<br><br>Pull-out test | Partial extrusion of EV sample from the skin after 3 days.<br><br>No visible movements for the EVCP specimen.<br><br>↑ length of the protrusion for the EV from 3 mm to 9 mm after 14 days (60% of extrusion).<br><br>No protrusion for EVCP | The HA layer favored the integration with the surrounding skin tissue preventing epidermal downgrowth and pocket formation<br><br>The immobilized laminin further improved the adhesion strength between the film and the skin | Oyane, 2011 |

| | | | specimens | | |
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| | | | Epidermidis migration from 0.92 to 2.40 mm with a pocket formation up to 2.13 mm in depth between the specimen and the epidermis for EV | | |
| | | | Direct contact among epidermidis, derma, subcutaneous tissue and specimen for EVCP with a slight downgrowth (0.20–0.30 mm) and pocket formation (0.08–0.24 mm) | | |
| | | | Direct contact among EVLCP and epidermal, dermal, and subcutaneous tissues with slight downgrowth (0.19–0.22 mm) and pocket formation (0.03–0.17 mm). | | |
| | | | ↑ maximum load to pull out ECLCP *vs* EVCP specimen | | |
| Twelve rats underwent to the dorsal implant in which the device was implanted subcutaneously leaving the post to protrude through the skin.<br><br>Animals received gauze and semi-occlusive base dressing.<br><br>Animal were divided into two | Machined smooth titanium alloy (Ti6Al4V) percutaneous post, and subdermal component with a Ti6Al4V core covered with commercially pure titanium porous coating (K-coating) | Histology<br><br>Immuhistochemistry<br><br>Downgrowth measurements | No signs of infection in both groups<br><br>No statistical difference in downgrowth at 4 weeks between NPWT group (2.6 ± 3.5%) and untreated group (5.3 ± 5.1%)<br><br>↑ blood vessel density in the wound edge of the NPWT Group (1.6×) *vs* untreated Group.<br><br>↑ blood vessel density in both | NPWT reduced the epidermal downgrowth compared to the Untreated Group.<br><br>NPWT can increase blood vessel densities twofold compared to untreated tissues. | Pawar, 2019a |

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| groups:<br><br>Negative Pressure Wound Therapy (NPWT) group treated with NPWT set to apply −70 to −90 mmHg using a vacuum pump<br><br>untreated group.<br><br>4 weeks | | | NPWT and untreated groups (6.1× and 3.4×, respectively)<br><br>No significant difference in macrophages between<br><br>untreated and NPWT Groups.<br><br>40% of the sealed interface in the untreated group with the epidermis *vs* 100% of the skin-implant sealed | | |
| Twenty-five rats implanted with devices in the subcutaneous tissue and sutured to the surrounding skin<br><br>Euthanasia at 4 weeks | Commercially pure<br><br>Ti rods and meshes<br><br>with fiber diameter of 120 um and mesh spacing of 213 um.<br><br>Four types of implant:<br><br>- TI,<br>- HA coated Ti,<br>- TI-Mesh (a Ti mesh covering the grove of Ti), and<br>- HAMesh (Ti mesh coated with HA).<br>the thickness of the coating layer of HA was 3–5 um.<br><br>TI-Mesh was a Ti specimen having a mesh covering a groove, 3 mm in width and 0.5 mm in depth, machined on its lateral surface | Histology<br><br>Mechanical tests | All Ti specimens detached from the skin in 3-14 days (0% survival rate);<br><br>all HA specimens detached in 7-16 day (0% survival).<br><br>1/15 Ti-Mesh detached at day 6 (93% survival) and 0/15 HA-Mesh detached (100%survival)<br><br>↑ survival rate for TI- and HA-mesh *vs* TI and HA specimens.<br><br>No significant differences in peak load and attachment strength between Ti- and HA mesh<br><br>Similar histological findings between TI- and HA-Mesh<br><br>no significant downgrowth of the dermal tissue | The mesh structure seems to represent a candidate for the mechanical attachment of the connective tissues | Asoda, 2013 |

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| Twelve rabbits submitted to amputation.<br><br>Three groups:<br><br>Group 1: non-porous solid Ti transcutaneous component;<br><br>Group 2: porous Ti transcutaneous component;<br><br>Group 3: porous Ti transcutaneous component seeded with autologous fibroblasts in collagen gel<br><br>Six-eight weeks after the intraosseous component implantation, skin was opened and the transcutaneous porous component was connected to the intramedullary component<br><br>After 2 months one animal per group was evaluated histologically.<br><br>5 months | Transcutaneous component: a threaded implant surrounded by a porous cladding in sintered titanium alloy.<br><br>The cladding had an average range of porosity of 45–50% with pore size in the range of 60– 120 μm.<br><br>Prior to the in vivo implant, the device in Group 3 was cultivated for 7 day in collagen gel enriched with autologous fibroblast obtained from skin fragment | Histology<br><br>Bacteriological assay | In Group 1 no attachment of the dermal or subdermal tissues to the implant surface and detection of marsupialization phenomenon. Infective complications in all animals of Group 1 related to S. Aureus and E. Coli, with the development of osteomyelitis and loosening of the intramedullary implant.<br><br>In Group 2 attachment of the derma to the porous component after 6–7 weeks in all rabbits and no infectious complications.<br><br>In Group 3, the attachment of the derma was observed at 3–4 weeks, No signs of infection in any of the animals. | Fibroblast seeding in a collagen matrix enhances ingrowth of dermal and subdermal soft tissues into the implant surface *vs* unseeded implant | Shevtsov,2014 |
| Nine rabbits underwent to percutaneous implant in the | Ti nanotubes (TNT) with 100 nm diameters obtained | Antibacterial assay | ↓ antibacterial activity for PolTi while ↑ for TNTs with highest | The nanostructured surface can improve | Tan, 2015 |

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| dorsum by creating subcutaneous pockets in which 3–5 mm of implant exposed.<br><br>After 4 hrs from surgery, animals received bacterial inoculations (S. aureus liquid, 108 CFU ml−1) directly to the skin/implant interface in the pockets.<br><br>2, 4 and 8 weeks. | with anodization technique in comparison with PolTi for the antibacterial evaluations | SEM<br><br>Immersion test<br><br>Histology and inflammatory factors detection | antibacterial activity after 7 days.<br><br>↓ bacteria at SEM on TNT surface *vs* PolTi<br><br>At 2 weeks no signs of inflammation at implant sites with injected of S. aureus *vs* fester manifestation around PolTi implant.<br><br>The PolTi implant injected S. aureus liquid released the ↑ amount of TNF-alpha and IL-1alpha, whereas TNT had the lowest value.<br><br>↓ release of cytokine in TNT than PolTi with or without injected S. aureus.<br><br>Tightly skin adhesion to TNT and poor integration with PolTi<br><br>Inflammatory responses in tissue injected with bacteria with fibrotic capsule formation: thinner in TNT than that induced by PolTi. | the cellular and tissue adhesion than PolTi | |
| Eight rabbits underwent to dorsum subcutaneous implant of the devices with the protruding post. Several implant combinations were evaluated:<br><br>1) smooth post/smooth subcutaneous disk (S/S),<br>2) smooth post/porous subcutaneous disk (S/P), | Ti6Al4V implants composed of a cylindrical percutaneous post and a subcutaneous disk with a porous surface coating with average pore size of ~400 µm and porosity of ~60%,*vs* polished surface | Microbiological investigations<br><br>Histology | ↓ risk of infection of 80% in the P/P implants and 77% in the P/S *vs* S/S implants. No infection in S/P group.<br><br>↑ risk of infection of 7-fold in S/S implants *vs* to porous coated implants<br><br>No statistical differences for epidermal downgrowth among | Incidence of infection is significantly reduced in porous surface especially with porous subcutaneous component | Isackson, 2011 |

| | | | implant type | | |
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| 3) porous post/ smooth<br>4) subcutaneous disk (P/S),<br>5) porous post/porous subcutaneous disk (P/P)<br><br>Six animals received weekly bacterial inoculations at the skin/implant interface of S. Aureus.<br><br>Two animals serve as a baseline control<br><br>14 weeks | | | Fibrovascular tissue in the porous subcutaneous disks while no tissue infiltration in porous percutaneous posts.<br><br>Mild or moderate inflammatory cells infiltration in porous structures. Moderate neovascular formation and adipose tissue infiltration in the pores<br><br>Fibrovascular tissue capsule in the smooth subcutaneous implant. Mild to moderate inflammatory reaction and minimal adipose tissue formation with mild neovascular presence | | |
| Forty rabbits in which a percutaneous distal tibial implant was performed<br><br>Coated rods were compared with uncoated rods.<br> At the time of implant, the percutaneous site was contaminated with 1 x 107 (CFU/mL) of S. aureus<br><br>1, 2 and 4 weeks | Sandblasted stainless steel 316L rods with aluminum oxide powder coated with a sol-gel containing 0% or 20% triclosan | Histology<br><br>Immunohistochemistry | Animals treated with coated pins showed no infection at all three time points.<br><br>Animals in the uncoated group showed high infection rates at all-time points.<br><br>↓ pocket depth at 4 weeks in the coated groups (<0.2 mm) *vs* uncoated group (5.1 mm).<br><br>↑ epithelial downgrowth in uncoated group with subcutaneous inflammation and abscess formation at all-time points. Immunostaining reveals the presence of S. aureus. | The incorporation of a bactericidal agent was effective in preventing infection development favoring a normal healing, bone tissue growth<br><br>with no epithelial downgrowth events. | Qu, 2015 |

| | | | No staining for bone markers (type I collagen, alkaline phosphatase and osteocalcin).<br><br>Positive staining for bone markers (osteocalcin, type I collagen and alkaline phosphatase in osteoblasts and the bone matrix) at 1 and 2 weeks in the coated group.<br><br>At 4 weeks bone integration and strong staining | | |
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| Twenty hairless guinea pig in which subdermal pocket was created on the dorsum in which the device was implanted leaving the post to protrude through the skin.<br><br>Animals were divided into four groups according to the type of subdermal coating:<br><br>Group 1 (control 1) animals were implanted in a One-Stage surgery with no NPWT;<br><br>Group 2 (control 2) animals were implanted in Two-Stage surgery with no NPWT; | The device (subdermal barrier and percutaneous post) was realized with Ti6Al4V.<br><br>Subdermal barrier was porous coated with commercially pure titanium (1 mm in thickness; K2 type; smal pore size ~30–55 μm; large pore size ~230–500 μm). | Clinical and histological evaluations | No signs of clinical infection<br><br>The mean downgrowth rate for group was:<br><br>Group 1: 28 ± 7%<br><br>Group 2: 8 ± 7%<br><br>Group 3: 16 ± 7%<br><br>Group 4: 5 ± 4%<br><br>All interfaces showed hyperplastic epithelium over a granulation type tissue.<br><br>No migrating epidermal layer to the interfaces in group 1<br><br>In group 2, 4/4 implant had migrating epidermal layer<br><br>In group 3 and 4, 3/5 had migrating epidermal layer. | The NPWT seems to limit the downgrowth rate regardless of the surgical approach<br><br>The two-Stage surgical approach seems to reduce the downgrowth rate (-36%) vs the one-Stage. | Mitchell, 2016 |

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| Group 3 One-Stage surgery with NPWT;<br><br>Group 4 animals with Two-Stage surgery and NPWT.<br><br>4 weeks after surgery in groups 1 and 3<br><br>3 weeks in groups 2 and 4 from the first and 4 weeks after the second surgery<br><br>NPWT application after the implantation of the percutaneous posts. Negative pressure regimen of 280 to 2100 mmHg administered for approximately 108 hours/weeks with a cycle of 36 hours followed by 12 hours of no therapy. | | | | | |
| Twenty hairless guinea pig in which subdermal pocket were created on the dorsum in which the device was implanted leaving the post to protrude through the skin.<br><br>Animal were divided into six groups according to the type | Device with a subdermal component in titanium alloy (Ti6Al4V) porous coated with commercially pure titanium (K2 Type), and a smooth percutaneous post | Clinical and histological evaluations | Mean downgrowth without NPWT was 22.4%*vs* mean downgrowth rate with NPWT 6.9%<br><br>The mean downgrowth in group 1 was 22.4% while the use of NPWT (group 2) reduced this value to a 6.9%<br><br>The mean downgrowth rate for | For all device types, NPWT reduced downgrowth.<br><br>HA was more effective in limiting downgrowth<br><br>NPWT and HA | Jetapalina, 2019 |

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| of subdermal coating:<br><br>1) Porous coated titanium (historical samples);<br>2) Porous coated titanium with negative pressure wound therapy (NPWT) application (historical samples);<br>3) Collagen type I foam coating;<br>4) Collagen type I foam coating with NPWT;<br>5) Plasma spray HA coating with 62% crystallinity with a thickness of 10 μm and<br>6) HA coating associated with NPWT<br><br>28 days with a pressure regime of −80 to −100 mmHg for 36 h of continuous therapy followed by 12 h of rest. | | | no coating was 17.7%, for collagen coating was 18.5% and for HA coating was 8.4 %<br><br>Epithelial cells with well-defined, differentiated layers with the association between biomimetic coatings and NPWT | biomimetic coating independently and in association limit the epithelial downgrowth | |
| Six guinea pigs submitted to implantation of the subdermal component under the dorsal skin in a first stage surgery.<br><br>Three weeks after, in a second surgery, the percutaneous post was connected to the subdermal device.<br><br><br>Animals received a dressing | Subdermal component and percutaneous post of Ti6Al4V.<br><br>The subdermal device was further treated with commercially pure Ti porous coating (K-coating) with pore size distribution of 230–500 um. | Histological analysis | ↑ downgrowth in the Discontinued Group (23± 3%) *vs* NPWT Group (5±4%). No differences *vs* untreated Group (16±6%)<br><br>↓ vascularization in Discontinued *vs* NPWT Group. No differences in comparison with untreated<br><br>↑ fibrosis in the granulation tissue in peri-prosthetic area for the Discontinued and Untreated *vs* the NPWT treated tissues.<br><br>Acanthosis in each group at the | The data available suggest that NPWT only limited downgrowth while being applied.<br><br><br>4 weeks after discontinuing<br><br>NPWT, the amount of epithelial downgrowth was comparable<br><br>to the Untreated | Pawar, 2019b |

| | | | 3- point junction with no differences among the three group. | Group after 4 weeks, without NPWT | |
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| placed over the incision and the percutaneous post, covered and secured into | | | | | |
| a semi-occlusive dressing fashion. | | | ↑ acanthosis in the NPWT and | | |
| | | | Untreated Groups *vs* Discontinued Group | | |
| A NPWT treatment was settled for a continuous apply of 80–90 mmHg of vacuum for 4 weeks. | | | | | |
| After that the treatment was discontinued. | | | | | |
| Four weeks after discontinuation the | | | | | |
| animals were euthanized. | | | | | |
| 8 weeks after second stage surgery. | | | | | |
| This group is referred to as the "Discontinued Group" that was compared with previous published groups in which the NPWT was administered for 4 weeks (NPWT treatment group) and to Untreated Group that received no treatment for 4 | | | | | |

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| weeks. | | | | | |
| Four swine were implanted with porous tantalum implants *vs* solid Ti implants in dorsal subcutaneous pocket with a post protruding through the skin.

42 day | Solid Ti core surrounded by a porous tantalum structure with 80% of porosities with dodecahedral interconnecting cells with average open pore diameter of 450 um realized as a post and implant base.

Vs polished solid Ti alloy control implants | Semi-quantitative score for epidermal contact (0 for no contact up to 2 for contact on both side of the implant post)

Penetration score for soft tissue and vascular tissue of 0-4 for the post (0 = no penetration; 1 = penetration of 1-25% of the distance to the titanium post; 2 = penetration of 26-50% of the distance to

the titanium post; 3 = penetration of 51-75% of the distance to the titanium post; and 4 = penetration of 76-100% of the distance to the titanium post9.

A penetration score was also assigned to each implant at the

implant base (0-4).

Semi quantitative evaluation of the chronic and acute inflammation | 16/18 (88%) of porous tantalum implants integrated with the soft tissues and in situ. 1/18 partially extruded and 1/18 completely extruded.

3/4 of the solid Ti implants completely extruded and 1/4 remained in situ.

Overall, 9/16 direct epidermal contact at the skin/post interface. 6/9 contact at both side of the implant while 2/9 unilateral contact.

The solid Ti implant did not exhibit any dermal contact, soft tissue penetration or vascular growth.

15/16 soft tissue penetration (94%) and vascular permeation in porous implants in the post (6/16) and in the base (15/16). The post score was 1.25 and 2.63 for the base and 5/16 exhibited vascular ingrowth at the post level with a score of 1. | Porous tantalum implants seem to be able to favor soft tissues penetration at dermis and subcutis levels | Hugate, 2015 |

| | | (0-4, where 0 = finding not present, 1 = minimal, 2 = mild, 3 = moderate, and 4 = marked). | Score for acute inflammation was 1.88 at the post in the porous implant and 0.81 at the base. Chronic inflammation was present at the post implant in porous implants (score of 1.5) and at the base (1.56). In the Ti implant, no detection of acute or chronic inflammation at the post and at the base (score: 2) | | |
| Two pigs in which the devices were implanted in subcutaneous pockets with pin partially protruding through the skin<br><br>One-week post implantation one animal received Vaseline® treatment around percutanoeus implant to apply a Tran-Epidermal Water Loss (TEWL) (treated group) vs no Vaseline® (untreated group)<br><br><br>12 weeks | Porous Ti coated device with a percutaneous and a subdermal component in a solid configuration or with a perforated surface (1.5 mm diameter holes). The sub-dermal component and lower portions of the pin were coated with 1 mm thick, Ti porous coating. | Histology<br><br><br>Immunohistochemistry | Skin regression at the interface in both treated and untreated implants.<br><br>↑ surface coverage with sub-epithelial tissue for the untreated group (77.8 ± 1.8%) vs treated group (36.5 ± 3.3%)<br><br>↑ cutaneous regression in treated group<br><br>↑ expression of molecules related to inflammation and peri-implant tissue hyperplasticity | TEWL management may be contraindicated for the maintenance of the skin-implant interface of percutaneous devices. | Holt, 2013b |
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| Two bred cats submitted to amputation to distal level of the tibia followed by the implant of the intramedullary component<br><br>21 weeks from the implant surgery. | Porous composite implants constituted by sintered Ti particles and solid inserts (Ti6Al4V). The porous component had the following characteristics: pore size (40–100 µm), particle size (100–200 µm), porosity (30–50%) and volume fraction (40–45%). | Histological evaluation of the skin ingrowth into the implant | No skin infection or signs of pain or distress.<br><br>The epidermal layer growth into the pores up to the deeper porosities.<br><br>Dermal ingrowth was assessed in one animal for technical reasons.<br><br>Skin penetrated the implant to a depth of approximately 0.7 mm and followed the edge of the implant. | Porous Ti implants may permit skin integration | Farrell, 2014a |
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| Four dogs submitted to amputation for malignant neoplasia of the distal limbs | Custom device manufacturing for each animal made of Ti6Al4V stem for the intramedullary component, a perforated umbrella-shaped flange for the subcutaneous implant and a distal extracutaneous peg as connector between the stem and flange portion and the exoprosthesis.<br><br>The base of the stem and the flange was plasma sprayed with Ti (70- to 100-µm thickness) and subsequently treated with HA (50–70 µm) to provide a porous surface. | Histological examination of one the four implants at euthanasia due to metastatic dissemination of the tumors one year after surgery | Incorporation of the flange in dermal tissue with the epidermis abutting onto the stem of the implant.<br><br>Epidermis downgrowth along the stem of around 3 mm.<br><br>Epithelium attachment to the HA-coated surface with keratinized epidermidis.<br><br>No fibrotic encapsulation of the flange.<br><br>Fibroblast colonization of flange | Relevant data for the clinical planning of this type of device | Fitzpatrick, 2011 |

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| | The flange was perforated by holes of 0.7 mm diameter.<br><br>The external peg was treated with low surface energy "diamond-like carbon coating, DLC" (2- to 4-µm thickness) | | | |

**ABBREVIATIONS**

Titanium = Ti; Hydroxyapatite = HA; Adorbed = Ad; Fibronectin = Fn; Polished = Pol; Silanized = Si; Silver = Ag; Micro-Arc Oxidation = MAO; Alkali Heat Treatment = AHT; Hydrothermal Treatment = HT; Fibroblast Growth Factor = FGF; Mesenchymal Stromal Cells = MSC; Negative Pressure Wound Therapy = NPWT; Ti Nanotubes = TNT; Tran-Epidermal Water Loss = TEWL.