surgeries

MDPI

Review

From Basic Science to Clinical Perfection: What Defines the Orthopedic Biocompatible Implant?

Andrei Marian Feier [1,2], Tudor Sorin Pop [1], Paul-Gabriel Borodi [2,*], Sándor-György Zuh [1], Andrei Oprișan [1,2], Octav Russu [1] and Tiberiu Bațagă [3]

[1] Department of Orthopaedics and Traumatology, Clinical County Hospital, 540139 Targu Mures, Romania
[2] Faculty of General Medicine, University of Medicine, Pharmacy, Sciences and Technology, 540139 Targu Mures, Romania
[3] Clinic of Orthopaedics and Traumatology, County Clinical Emergency Hospital of Targu Mures, 540136 Targu Mures, Romania
* Correspondence: borodi.paul@yahoo.com; Tel.: +40-746349783

Abstract: The general improvement in life expectancy and standard of living makes it easier for patients to get access to routine medical exams and is anticipated to increase the prevalence of several degenerative joint illnesses. In addition, it is anticipated that their incidence will increase both nationally and internationally, which will raise the demand for novel and long-lasting implantable devices in the field of orthopedics. The current review's goals are to define what constitutes a biocompatible orthopedic implant in terms of in vitro biocompatibility testing and to clarify important concepts and definitions that are already in use. The demand for materials and implants made of various tissues is now increasing, and the ongoing advancement of in vitro cell culture studies is a reliable practical tool for examining the biocompatibility of potential implantable materials. In vitro biocompatibility research has been reduced and, in most cases, diminished to laboratory studies that no longer or drastically reduce animal sacrifice as a response to the well-known three "Rs" ("reduction", "refinement", and "replacement") introduced to literature by English academics in the 1960s. As technology advances at an astounding rate, a new generation of gene-activating biomaterials tailored for specific people and disease conditions might emerge in the near future.

Keywords: biocompatibility; implant; degenerative joint diseases

Citation: Feier, A.M.; Pop, T.S.; Borodi, P.-G.; Zuh, S.-G.; Oprișan, A.; Russu, O.; Bațagă, T. From Basic Science to Clinical Perfection: What Defines the Orthopedic Biocompatible Implant? *Surgeries* **2023**, *4*, 1–9. https://doi.org/10.3390/surgeries4010001

Academic Editor: Cornelis F.M. Sier

Received: 1 October 2022
Revised: 29 November 2022
Accepted: 7 December 2022
Published: 27 December 2022

1. Introduction

An increasing number of diagnosed degenerative joint pathologies in the foreseeable near future is projected [1]. General increase in life expectancy and quality of life facilitates patients access to regular medical examinations and are expected to increase the prevalence of certain degenerative joint diseases [2]. Secondary to this, a growth in their incidence both nationally and globally is also expected, bringing a rising demand for innovative and durable implantable devices in the field of orthopedics [3]. Currently, the prevalence of degenerative joint disease on the Eastern European continent is rising (13.4% in 2020) which drags demand for large-scale implantable devices [4]. A highlight in increasing prevalence of degenerative joint diseases in younger people is also anticipated. In addition to socio-economic and functional impact, there is the issue of properties related to mechanical strength, osseointegration and durability of the implant. Enhancing various properties of a specific implant requires a multidisciplinary approach with aid from interconnection of different specialties such as: material engineering, cell biology, orthopedic surgery and not only [5].

Presently, the world is endorsing a growing need for materials and implants of various tissues and the permanent development of cell culture in vitro studies is an upright practical instrument for investigating the biocompatibility of future implantable materials. As a response to the well-known three "Rs" ("reduction", "refinement" and "replacement")

brought to literature by English academics in the 1960s, in vitro biocompatibility research has been reduced and, in most cases, diminished to laboratory studies that no longer or drastically reduce animal sacrifice [6].

The purpose of the current review is to establish what defines the biocompatible orthopedic implant in terms of in vitro biocompatibility testing and clarify key concepts and definitions available today.

2. Biomaterial: Brief History and Definition

There are plenty of historical descriptions and reports of procedures for introducing different types of devices in the human body [1,2]. These ancient reports include procedures performed for replacing teeth, different bony structures or wound regeneration attempts [3]. However, biocompatible materials did not exist as we distinguish them today. Throughout history, the word "biomaterial" per se was not used in academic language and it was reported under distinctive names. The term was mostly synonymous with "implantable device", "prosthesis", "material augment", etc. In the mid-18th century, as scientific communities became more robust and industrialized, the area of implantable materials gained additional popularity.

It was in the middle of 19th century when conferences and scientific gatherings around the world began methodically focusing on implantable devices and their usage as replacements in different anatomic parts. The first *"almost-definition"* of a biomaterial was made in 1967 by pioneer orthopedic surgeon Jonathan Cohen [4]. He defined all materials (metals, bone and derivatives used as bone grafts, plastics ceramics and composites) as "biomaterials" excluding drugs and fabrics used for sutures [5].

Only two years later, several symposiums were organized focusing predominantly on materials and their use for reconstructive surgery. Society For Biomaterials was founded by Dr. William Hall and his colleagues in 1974 with the aid of visionary bioengineers from Clemson University [6]. Therefore, a newly emerged organization was established and was set to accurately institute a new definition for the concept of biomaterials: *"A biomaterial is a systematically, pharmacologically inert substance designed for implantation within or incorporation with a living system"* [7].

Further on, a definition published by British professor David F. Williams gained wide criticism throughout previous decades [8,9]. He stated that a biomaterial is "a nonviable material used in a medical device, intended to interact with biological systems" [10]. It not only lacked explanations made on what precisely "nonviable" means, but the exclusion of biological tissues (bone grafts, tendons, ligaments, etc.) combined with various pharmacological products conveyed numerous controversies in the literature. As of today, half a century after the first attempts, the definition does not seem too far away but definitely multidisciplinary. The latest definition was proposed in United Kingdom in 1986 and approved afterwards in 1991 in a proceedings paper of Consensus Conference held by the European Society for Biomaterials: *"Any substance or combination of substances, other than drugs, synthetic or natural in origin, which can be used for any period of time, which augments or replaces partially or totally any tissue, organ or function of the body, in order to maintain or improve the quality of life of the individual"* [11].

The state-of-the-art definition agrees clearly and analytically on its previous troubling mentions and avoids almost every bias. It is a well-defined traceable result of several multidisciplinary meetings, mutual agreements and pooled opinions.

3. The Orthopedic Biomaterial: State-of-the-Art Emerging Concepts

Up until a few decades ago, a new biomaterial introduced in the commercial lines of implantable devices manufacturers consisted of new bulk technologies such as: stents, wires, titanium cerclages, biodegradable screws and so on. Emerging concepts in present-day technology include extremely advanced biomaterials and include targeting nanocarriers with specially designed localized delivery systems [12]. In the field of orthopedics, implantology challenges emerge at the border of local reactions to metallic implants with

personalized implant surfaces and general inflammatory reactions as a result of host–implant response [13]. While characteristics and occurrences of implantable device allergies seem to be left aside [14], an in-depth breakdown of adverse local reactions and methods of improving bone–implant interface osseointegration seem to arise [15].

Another breaking topic of significance remains around improving implants surface at a micro and more innovative, at nano scale level. This mainly consists of coating specific surfaced areas of implants [16,17] (e.g., trochanteric region of femoral stem, femoral condyle region of knee prosthesis). These procedures are generally advancing with numerous in vitro studies and subsequently implemented in clinical trials [18]. A detailed look at the steps involved in defining a good biocompatible implant is described in Figure 1.

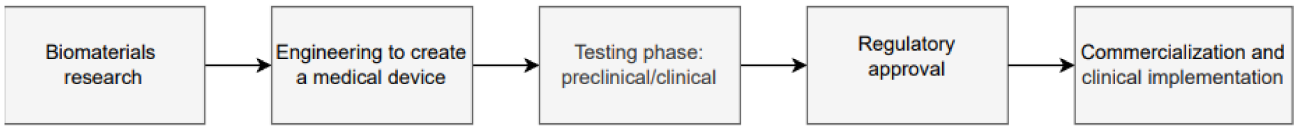

Figure 1. Steps that are mandatory to bring potentially innovative biomaterial technology to an implantable level.

Orthopedic surgeons usually quantify the performance of an implant based on the rate of early aseptic revision of the surgical intervention (e.g., revision total hip arthroplasty). This is certainly biased due to implant malposition and technique errors. An example of quickly emerging basic science into clinical science are hip arthroplasty liners infused with Vitamin E [18]. Based on this and with a primordial aim of reducing revision rates and increasing commercial power, implant manufacturers bring innovative in vitro ideas to in vivo. This latest addition of Vitamin E to hip replacement polyethylene liners was found to reduce revision rates by 46% in a short-term follow-up meta-analysis [19].

Another novel, promising category of biomaterials is osteoinductive materials. There are several methods for imparting osteoinductive properties into scaffolds: surface modification, inclusion of growth factors (transforming growth factor, morphogenetic proteins, endothelial growth factors, etc.) and stem cells deposits [20]. Several authors reported methods for dual-delivery of growth factors or other constituents into wounded areas with the final aim of stimulating bone formation [21]. Incorporating nanoparticles into the scaffold used for bone tissue engineering is yet another method that may be used to deliver growth factors in applications related to orthopedics. The encapsulation of proteins into nanoparticles, which are subsequently transported via scaffolds, would allow for more precise control of their release and would provide the long-term sustained release patterns sought for particular growth factors [22].

In the field of musculoskeletal medicine and orthopedics, scientists are continuously pursuing an ideal substrate biomaterial to use as a delivery system for therapeutic stem cell use [21]. Almost any type of damaged tissue in the human body is susceptible to regeneration processes and subsequent immune regenerative reactions. Regenerative approaches using stem cells are the diamond of our medical generation and continue to rise with every new basic science breakthrough [20]. Stem cell encapsulation is a novel concept in which a population of cells are restrained and temporarily blocked inside a biocompatible matrix substrate [22]. The substrate allows for O_2 and metabolites to pass through and provides several benefits [23]. It is currently used in minimally invasive cartilage procedures for delivering collagen and hyaluronic acid molecules to specific areas intra-articularly. Delivery includes a controlled and continuous supply of therapeutic agents and protection from host–immune cell reactions [24]. Bone specific clinical use includes bioactive constituents filled with encapsulated stem cells able to fill bone defects after trauma, tumor removals or revision arthroplasties [24].

It is undeniable that tissue engineering, combined with suitable, thoughtful basic science ideas and lab studies that are consequently applied clinically are the future of biomedicine.

4. From Laboratory to Clinical Practice: In Vitro Biocompatibility Testing

In the previous three decades, immense attention was ascribed to in vitro biocompatibility studies of novel biomaterials, consequently being detrimental to in vivo studies. An expectable outcome was a progress on cell-biomaterial interaction theories (cell adhesion, proliferation, viability, material roughness, surface adaptation, etc.) that resulted in a fast development of novel in vitro study models, products and their implementation in clinical practice [25].

After identifying the need for an improvement of a biomaterial, surface or feature, a clear description of novel mechanical properties is established. Cytotoxic testing commonly begins simultaneously with cell culture analysis in vitro and according to several authors they are followed by fluorescent staining of different types [26]. Culture testing implies an in-depth analysis of protein interactions and synthesis, cell viability, adhesion and proliferation processes [27]. Several particularities of each step involved are described in detail in Figure 2.

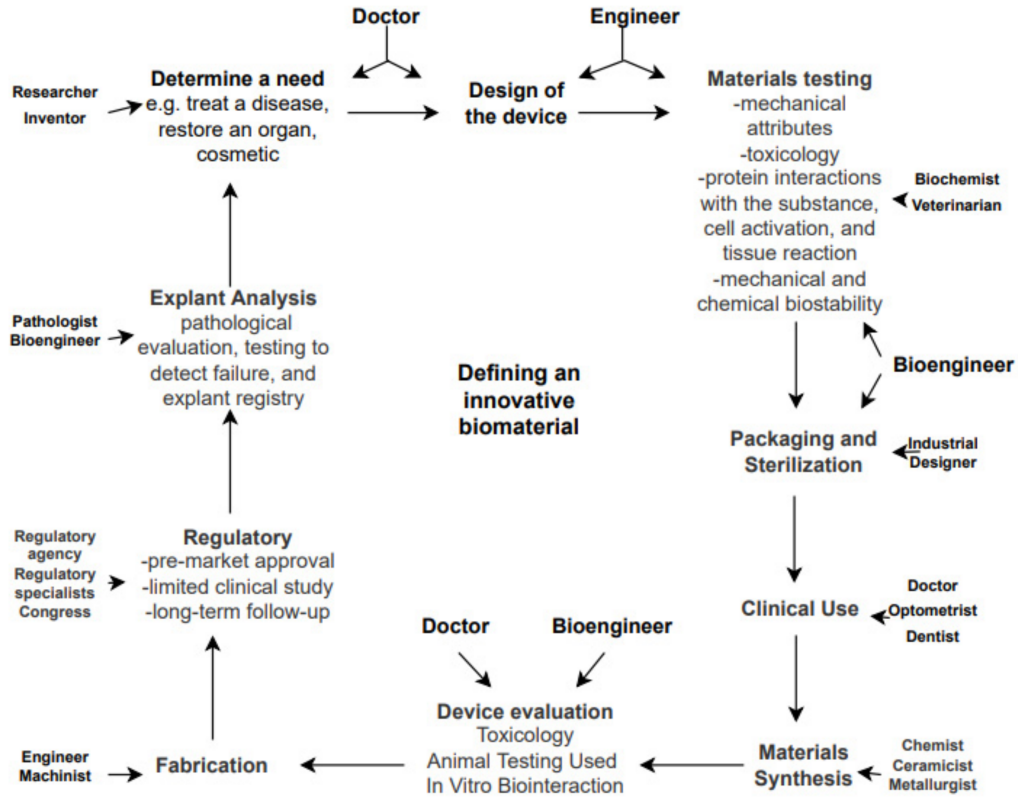

Figure 2. Disciplines involved in biomaterials science and the path from a need to a manufactured medical device.

Three main types of cells used are: tumor-derived osteoblastic cells, primary cells and commercial lines.

4.1. Human Bone Derived Cells: Osteoblasts Behavior Analysis on Biomaterials

There are three main common types of cells sources used for biocompatibility testing: tumor-derived osteoblastic cells, primary cells and commercial lines [28]. A summary of their differences and advantages can be seen in Table 1.

Human derived bone cells (HDBCs) are considered a contemporary gold standard for in vitro biocompatibility studies [29]. Particularly, primary bone-derived osteoblasts (OB) are key in obtaining outcomes that almost entirely biomimic in vivo microenvironments [30].

Table 1. The main common types of cells sources used for biocompatibility testing. Differences and advantages.

Commercial Osteoblasts Lines		**Primary Osteoblast Cultures**	
Cons	**Pros**	**Cons**	**Pros**
minimal relevance in vivo	require standard culture medium	reduced contamination	thorough ethical regulations
genomic inconsistencies	clear morphological characteristics	maintains the phenotype after passages	low availability
general contamination	standardized ethical regulations	high relevance in vivo	require specific culture medium and specific components
contamination with elements of various cells	high availability	genetically stable	low costs
high costs	unlimited supply	usable in personalized medicine	lack of standard morphological characteristics

Primary cells are subject to environmental variables and adjust their phenotype and genotype accordingly, whereas commercial lines phenotype is standardized frequently, resulting in biased outcomes on cell cultures [31]. OB have the ability to proliferate, adhere, multiply and stimulate mineralization processes on tested substrates [32]. Three important indicators for cell health are their attachment, confluence and focal adhesions. These properties are commonly evaluated by using a combination of optical and confocal scanning microscopy. Tribological properties are thus quantified at a quantitative and qualitative level and provide insights close to comprehension regarding biocompatibility of tested materials.

OBs behavior is primarily influenced by the physical and chemical properties of the substrate. Remarkably, Lamers et al. have proved that substrate nano-level morphology (width, depth, spacing) controls OB behavior, showing enhancement in their motility [33]. Emerging novel concepts in implant surface modifications will drastically improve osseointegration and implant durability.

4.2. Orthopedic Implant Osseointegration: Improvements, Innovations and Foresights

An osseointegrated viable implant is defined by its ability to provide direct structural and functional connection between the bone and a biomaterial or implant at the interphase level [28,34]. A widely accepted definition of osseointegration is established by American Academy of Implant Dentistry: *"Contact established without interposition of nonbone tissue between normal remodeled bone and an implant entailing a sustained transfer and distribution of load from the implant to and within the bone tissue"* [35]. With recent discoveries on dental implantology, it is now known that three central biological phases begin at bone-to-implant contact: inflammatory, proliferative and maturation phase [36]. Both surface microroughness and nanoroughness influence variables of in vitro cell behaviors that modulate aforementioned phases [37]. The microsurface roughness modifications are currently often performed using 3D printing technologies [38]. This enables engineers to construct grafts and scaffolds that can be organized at a level similar to environmental conditions in vivo. In addition, materials engineering advanced technologies such as electrochemical anodization, electrospinning or hydrothermal treatments allow researchers to organize roughness and topography at a nanoscale level [39–41]. While these methods allow a better structure of implant surface, they also improve osseointegration by allowing bone cells to develop their actinic cytoplasmic extensions inside the scaffolds. Furthermore, this significantly improves cell adhesion and finally improves osseointegration. A porous surface obtained by 3D printing was demonstrated to have higher biocompatibility biological responses when tested in vitro and compared to basic orthopedic titanium [39,42].

Over the past decade progress was reported by many authors that brought novel and undiscovered theories [43]. Even if osseointegration has a known biologic course, the surgical technique has a major role in obtaining optimal endosseous integration and proper implant placement. While clinicians still debate the gold standard in assessing proper implant osseointegration, there is no standard radiological or clinical protocol. Nonetheless, researchers seem to have developed a rabbit model that allows for preclinical assessment of osseointegration of dental implants [44].

Computer-assisted surgical guides and templates have been proven to improve precision of both dental and knee implant placements [36,37] but with increased expenses. Another innovation at biomaterial bone-contact interphase is the addition of physico-mechanical techniques that alter implant surface and provide it with hydrophilic properties.

4.3. Novel Biomaterials in Clinical Practice: Difficulties and Concerns

Implementing new biomaterials in day-to-day practice is a concern of young researchers and consequently of manufacturing companies. The primary goal sought should always be to improve end-product outcomes for patients. Increased effectiveness, less adverse effects, and lower costs should all be primary considerations when trying to design a successful biomaterial. However, economic matters and big companies partake in deciding what technologies make it to clinical practice.

Steps that are mandatory to bring a potentially innovative biomaterial technology to an implantable level are clearly regulated by organizations depending on territorial laws (e.g., ISO10993-1 for United States and Regulation 2017/745 for European Union) [45,46]. Due to the difficulty and painstaking effort required to complete each of these stages, numerous private businesses provide advisory services on how to design, define, and present a novel implant to the market. In order to measure the penetration, absorption, and local distribution of certain chemicals, specific protocol studies are created based on the class and type of medical device, the formulation, and the intended application. Figure 3 highlights some of these protocols' most important features.

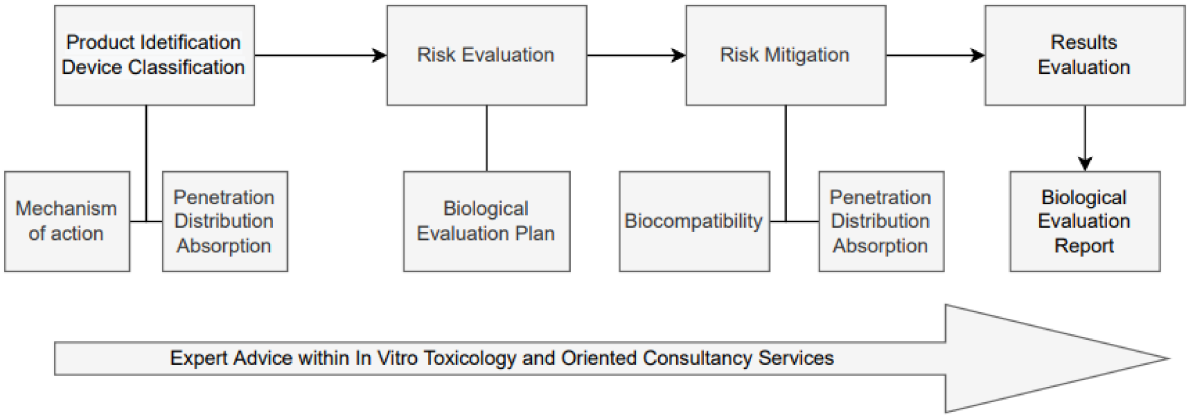

Figure 3. Steps that are mandatory to bring a potentially innovative biomaterial technology to an implantable level.

5. Animal Models and In Vivo Biocompatibility Testing

Animal tests, usage and mortality with the purpose of testing new viable biomaterials should be reduced as much as possible [47]. The three "Rs" ("reduction," "refinement," and "replacement") should always be the starting rule nowadays. However, toxicity and carcinogenity of specific materials must be assessed as part of a biological evaluation plan.

In vivo experiments usually begin with a proper selection of an animal model to be used. Each model has its advantages and disadvantages and the selection should take into account several variables, such as: target tissue type, material type, material chemical components, in vitro test results and local ethical restrictions or regulations. Goat and sheep models have been widely studied in the latest decades due to their bone size and

structure similarities compared to humans [48,49]. However, these relatively big sized models only make up to 1% of animals used in experimental research. Their breed is usually not specifically made for research and they require dedicated facilities for housing, surgical interventions and maintenance [50].

Other models, such as rodents, chicken and mice seem to dominate the scientific field in fundamental research and not only [51]. The DBA 1 strain of mice are commonly used to assess osteoarthritic changes and hypotheses related to skeletal degenerative processes. Nonetheless, these small animal models are commonly waived from cartilage research due to their frailty and vulnerability to minimal damage. Porcine models are known for lacking basic regenerative processes that may halt or bias end-results in research that implies bone loss or bone infections [52].

The end-goal of choosing an animal model is reaching a viable product that can be translated into clinical practice. The desired biomaterial target usage should be the starting point of a research hypothesis when preparing to choose the animal model. One should take into account both preclinical results obtained (both in vivo and in vitro) correlated with anatomical desiderates and local biomechanics.

6. Conclusions

As technology progresses at an astonishing pace, the near future should witness the development of a new generation of gene-activating biomaterials suited for certain individuals and disease states. A novel biocompatible orthopedic implant is defined as the sum of multiple and complex research outcomes that are the fundamental ground for translation to clinical environments.

Author Contributions: Conceptualization, A.M.F. and T.B.; software, A.M.F. and P.-G.B.; validation, T.S.P., S.-G.Z., O.R. and T.B.; resources, A.M.F., P.-G.B. and A.O.; writing—original draft preparation, A.M.F.; writing—review and editing, T.S.P., S.-G.Z., O.R., T.B., P.-G.B. and A.O. visualization, T.S.P., S.-G.Z., O.R. and T.B.; supervision, T.S.P. and T.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Ko, W.H. Early History and Challenges of Implantable Electronics. *ACM J. Emerg. Technol. Comput. Syst.* **2012**, *8*, 8. [CrossRef] [PubMed]
2. Madry, H.; Grässel, S.; Nöth, U.; Relja, B.; Bernstein, A.; Docheva, D.; Kauther, M.D.; Katthagen, J.C.; Bader, R.; van Griensven, M.; et al. The future of basic science in orthopaedics and traumatology: Cassandra or Prometheus? *Eur. J. Med. Res.* **2021**, *26*, 56. [CrossRef] [PubMed]
3. Shah, J.B. The history of wound care. *J. Am. Coll. Certif. Wound Spec.* **2011**, *3*, 65–66. [CrossRef] [PubMed]
4. Marin, E.; Boschetto, F.; Pezzotti, G. Biomaterials and biocompatibility: An historical overview. *J. Biomed. Mater. Res. Part A* **2020**, *108*, 1617–1633. [CrossRef] [PubMed]
5. Cohen, J. Biomaterials in orthopedic surgery. *Am. J. Surg.* **1967**, *1*, 31–41. [CrossRef]
6. Ratner, B.D.; Hoffman, A.S.; Schoen, F.J.; Lemons, J. *Biomaterials Science: A Multidisciplinary Endeavor*; Elsevier: Amsterdam, The Netherlands, 2013.
7. Park, J.B. Introduction. In *Biomaterials Science and Engineering*; Springer: Boston, MA, USA, 1984; ISBN 978-1-4612-9710-9.
8. Todros, S.; Todesco, M.; Bagno, A. Biomaterials and Their Biomedical Applications: From Replacement to Regeneration. *Processes* **2021**, *9*, 1949.
9. Zhang, K.; Ma, B.; Hu, K.; Yuan, B.; Sun, X.; Song, X.; Tang, Z.; Lin, H.; Zhu, X.; Zheng, Y.; et al. Evidence-based biomaterials research. *Bioact Mater.* **2022**, *25*, 495–503. [CrossRef]

10. Williams, D.F.; David, F. European Society for Biomaterials Definitions in biomaterials. In Proceedings of the Consensus Conference of the European Society for Biomaterials, Chester, UK, 3–5 March 1986; Elsevier: Amsterdam, The Netherlands; Chester, UK; Volume 4.
11. Doherty, P.J. Biomaterial-Tissue Interfaces. In Proceedings of the Ninth European Conference on Biomaterials, Chester, UK, 9–11 September 1991; Doherty, P.J., Ed.; Elsevier: Amsterdam, The Netherlands, 1992.
12. Din, F.U.; Aman, W.; Ullah, I.; Qureshi, O.S.; Mustapha, O.; Shafique, S.; Zeb, A. Effective use of nanocarriers as drug delivery systems for the treatment of selected tumors. *Int. J. Nanomed.* **2017**, *12*, 7291–7309.
13. Sheng, X.; Wang, A.; Wang, Z.; Liu, H.; Wang, J.; Li, C. Advanced Surface Modification for 3D-Printed Titanium Alloy Implant Interface Functionalization. *Front. Bioeng. Biotechnol.* **2022**, *10*, 850110.
14. Comino-Garayoa, R.; Cortés-Bretón Brinkmann, J.; Peláez, J.; López-Suárez, C.; Martínez-González, J.M.; Suárez, M.J. Allergies to Titanium Dental Implants: What Do We Really Know about Them? A Scoping Review. *Biology* **2020**, *9*, 404. [CrossRef]
15. Shin, Y.C.; Bae, J.-H.; Lee, J.H.; Raja, I.S.; Kang, M.S.; Kim, B.; Hong, S.W.; Huh, J.-B.; Han, D.-W. Enhanced osseointegration of dental implants with reduced graphene oxide coating. *Biomater. Res.* **2022**, *26*, 11. [CrossRef]
16. Hoskins, W.T.; Bingham, R.J.; Lorimer, M.; de Steiger, R.N. The Effect of Size for a Hydroxyapatite-Coated Cementless Implant on Component Revision in Total Hip Arthroplasty: An Analysis of 41,265 Stems. *J. Arthroplast.* **2020**, *35*, 1074–1078. [CrossRef]
17. Pap, K.; Vasarhelyi, G.; Gal, T.; Nemeth, G.; Abonyi, B.; Hangody, L.R.; Hangody, G.M.; Hangody, L. Evaluation of clinical outcomes of cemented vs uncemented knee prostheses covered with titanium plasma spray and hydroxyapatite: A minimum two years follow-up. *Jt. Dis. Relat. Surg.* **2018**, *29*, 65–70. [CrossRef]
18. Oral, E.; Wannomae, K.K.; Rowell, S.L.; Muratoglu, O.K. Diffusion of vitamin E in ultra-high molecular weight polyethylene. *Biomaterials* **2007**, *28*, 5225–5237. [CrossRef]
19. Cheng, Q.Y.; Zhang, B.F.; Wen, P.F.; Wang, J.; Hao, L.J.; Wang, T.; Cheng, H.G.; Wang, Y.K.; Guo, J.B.; Zhang, Y.M. Vitamin E-Enhanced Liners in Primary Total Hip Arthroplasty: A Systematic Review and Meta-Analysis. *Biomed. Res. Int.* **2021**, *6*, 3236679. [CrossRef]
20. De Witte, T.M.; Fratila-Apachitei, L.E.; Zadpoor, A.A.; Peppas, N.A. Bone tissue engineering via growth factor delivery: From scaffolds to complex matrices. *Regen Biomater.* **2018**, *5*, 197–211. [CrossRef]
21. Subbiah, R.; Hwang, M.P.; Van, S.Y.; Do, S.H.; Park, H.; Lee, K.; Kim, S.H.; Yun, K.; Park, K. Osteogenic/Angiogenic Dual Growth Factor Delivery Microcapsules for Regeneration of Vascularized Bone Tissue. *Adv. Healthc. Mater.* **2015**, *4*, 1982–1992. [CrossRef]
22. Wang, Z.; Wang, K.; Lu, X.; Li, M.; Liu, H.; Xie, C.; Meng, F.; Jiang, O.; Li, C.; Zhi, W. BMP-2 encapsulated polysaccharide nanoparticle modified biphasic calcium phosphate scaffolds for bone tissue regeneration. *J. Biomed. Mater. Res. Part A* **2015**, *103A*, 1520–1532. [CrossRef]
23. Shah, K. Encapsulated stem cells for cancer therapy. *Biomatter* **2013**, *3*, e24278. [CrossRef]
24. Hashemi, M.; Fatemeh, K. Application of encapsulation technology in stem cell therapy. *Life Sci.* **2015**, *143*, 139–146. [CrossRef]
25. Kulkarni, V.; Uttamani, J.R.; Asar, N.V.; Nares, S.; Tözüm, T.F. Evidence-Based Clinical Outcomes of Immediate and Early Loading of Short Endosseous Dental Implants: A Meta-analysis. *Int. J. Oral Maxillofac. Implants.* **2021**, *36*, 59–67. [CrossRef]
26. Czekanska, E.M.; Stoddart, M.J.; Ralphs, J.R.; Richards, R.G.; Hayes, J.S. A phenotypic comparison of osteoblast cell lines versus human primary osteoblasts for biomaterials testing. *J. Biomed. Mater. Res. A* **2014**, *102*, 2636–2643. [CrossRef]
27. Tomoaia, G.; Mocanu, A.; Vida-Simiti, I.; Jumate, N.; Bobos, L.D.; Soritau, O.; Tomoaia-Cotisel, M. Silicon effect on the composition and structure of nanocalcium phosphates: In vitro biocompatibility to human osteoblasts. *Mater. Sci. Eng. C* **2014**, *37*, 37–47. [CrossRef] [PubMed]
28. Harawaza, K.; Roach, C.P.; Fernandez, A. Modification of the surface nanotopography of implant devices: A translational perspective. *Mater. Today Biol.* **2021**, *12*, 100152. [CrossRef] [PubMed]
29. Carvalho, L.; Alberto, N.J.; Gomes, P.S.; Nogueira, R.N.; Pinto, J.N.; Fernandes, M.H. In the trail of a new bio-sensor for measuring strain in bone: Osteoblastic biocompatibility. *Biosens. Bioelectron.* **2011**, *26*, 4046–4052. [CrossRef] [PubMed]
30. Nasello, G.; Alamán-Díez, P.; Schiavi, J.; Pérez, M.Á.; McNamara, L.; García-Aznar, J.M. Primary Human Osteoblasts Cultured in a 3D Microenvironment Create a Unique Representative Model of Their Differentiation Into Osteocytes. *Front. Bioeng. Biotechnol.* **2020**, *8*, 336. [CrossRef]
31. Bernhardt, A.; Skottke, J.; von Witzleben, M.; Gelinsky, M. Triple Culture of Primary Human Osteoblasts, Osteoclasts and Osteocytes as an In Vitro Bone Model. *Int. J. Mol. Sci.* **2021**, *22*, 7316. [CrossRef]
32. Raut, H.K.; Das, R.; Liu, Z.; Liu, X.; Ramakrishna, S. Biocompatibility of Biomaterials for Tissue Regeneration or Replacement. *Biotechnol. J.* **2020**, *15*, e2000160. [CrossRef]
33. Lamers, E.; van Horssen, R.; te Riet, J.; van Delft, F.C.; Luttge, R.; Walboomers, X.F.; Jansen, J.A. The influence of nanoscale topographical cues on initial osteoblast morphology and migration. *Eur. Cell Mater.* **2010**, *9*, 329–343. [CrossRef]
34. Albrektsson, T.; Wennerberg, A. On osseointegration in relation to implant surfaces. *Clin. Implant. Dent. Relat. Res.* **2019**, *21*, 4–7. [CrossRef]
35. American Academy of Implant Dentistry. Glossary of implant terms. *J. Oral Implantol.* **1986**, *12*, 284–294.
36. James, L. Osseointegration: Its Mechanism and Recent Updates. *J. Dent Res. Pract.* **2022**, *4*, 1.
37. Golubewa, L.; Rehman, H.; Kulahava, T.; Karpicz, R.; Baah, M.; Kaplas, T.; Shah, A.; Malykhin, S.; Obraztsov, A.; Rutkauskas, D.; et al. Macro-, Micro- and Nano-Roughness of Carbon-Based Interface with the Living Cells: Towards a Versatile Bio-Sensing Platform. *Sensors* **2020**, *20*, 5028. [CrossRef]

38. Feier, A.M.; Portan, D.; Manu, D.R.; Kostopoulos, V.; Kotrotsos, A.; Strnad, G.; Dobreanu, M.; Salcudean, A.; Bataga, T. Primary MSCs for Personalized Medicine: Ethical Challenges, Isolation and Biocompatibility Evaluation of 3D Electrospun and Printed Scaffolds. *Biomedicines* **2022**, *10*, 1563. [CrossRef]
39. Kostopoulos, V.; Kotrotsos, A.; Fouriki, K.; Kalarakis, A.; Portan, D. Fabrication and Characterization of Polyetherimide Electrospun Scaffolds Modified with Graphene Nano-Platelets and Hydroxyapatite Nano-Particles. *Int. J. Mol. Sci.* **2020**, *21*, 583. [CrossRef]
40. Russu, O.M.; Strnad, G.; Jakab-Farkas, L.; Cazacu, R.; Feier, A.; Gergely, I.; Trambitas, C.; Petrovan, C. Electrochemical Synthesis of Nanostructured Oxide Layers on Threaded Surfaces of Medical Implants. *Rev. Chim.* **2018**, *69*, 1636–1639. [CrossRef]
41. Wang, J.; Liu, C.; Shen, S.G.; Wang, X.; Lin, K. The synergistic effect of 3D-printed microscale roughness surface and nanoscale feature on enhancing osteogenic differentiation and rapid osseointegration. *J. Mater. Sci. Technol.* **2021**, *53*, 18–26. [CrossRef]
42. Palka, K.; Pokrowiecki, R.; Krzywicka, M. Chapter 3–Porous titanium materials and applications. In *Titanium for Consumer Applications*; Elsevier: Amsterdam, The Netherlands, 2019; pp. 22–75.
43. Feier, A.M.; Manu, D.R.; Strnad, G.; Dobreanu, M.; Russu, O.M.; Portan, D.; Bataga, T. A Step Forward Standardization of Biocompatibility Testing on Tissue Culture Polystyrene. *Mater. Plast.* **2018**, *55*, 303–307. [CrossRef]
44. AlOtaibi, N.M.; Dunne, M.; Ayoub, A.F.; Naudi, K.B. A novel surgical model for the preclinical assessment of the osseointegration of dental implants: A surgical protocol and pilot study results. *J. Transl. Med.* **2021**, *19*, 276. [CrossRef]
45. Williams, D.F. On the mechanisms of biocompatibility. *Biomaterials* **2008**, *29*, 2941–2953. [CrossRef]
46. *Regulation EU. 2017/745 of the European Parliament and of the Council of 5 April 2017 on Medical Devices, Amending Directive 2001/83/EC, Regulation (EC) No 178/2002 and Regulation (EC) No 1223/2009 and Repealing Council Directives 90/385/EEC and 93/42/EEC*; OJ L 117, 5.5.2017; European Union: Brussels, Belgium, 2017; pp. 1–175.
47. Przekora, A.; Kazimierczak, P.; Wojcik, M. Ex vivo determination of chitosan/curdlan/hydroxyapatite biomaterial osseointegration with the use of human trabecular bone explant: New method for biocompatibility testing of bone implants reducing animal tests. *Mater. Sci. Eng. C* **2021**, *119*, 111612. [CrossRef] [PubMed]
48. Dias, I.E.; Viegas, C.A.; Requicha, J.F.; Saavedra, M.J.; Azevedo, J.M.; Carvalho, P.P.; Dias, I.R. Mesenchymal Stem Cell Studies in the Goat Model for Biomedical Research—A Review of the Scientific Literature. *Biology* **2022**, *11*, 1276. [CrossRef] [PubMed]
49. Dias, I.R.; Camassa, J.A.; Bordelo, J.A.; Babo, P.S.; Viegas, C.A.; Dourado, N.; Reis, R.L.; Gomes, M.E. Preclinical and Translational Studies in Small Ruminants (Sheep and Goat) as Models for Osteoporosis Research. *Curr. Osteoporos Rep.* **2018**, *16*, 182–197. [CrossRef] [PubMed]
50. Ribitsch, I.; Baptista, P.M.; Lange-Consiglio, A.; Melotti, L.; Patruno, M.; Jenner, F.; Schnabl-Feichter, E.; Dutton, L.C.; Connolly, D.J.; van Steenbeek, F.G.; et al. Large Animal Models in Regenerative Medicine and Tissue Engineering: To Do or Not to Do. *Front. Bioeng. Biotechnol.* **2020**, *8*, 972. [CrossRef]
51. Moran, C.J.; Ramesh, A.; Brama, P.A.J.; O'Byrne, J.M.; O'Brien, F.J.; Levingstone, T.J. The benefits and limitations of animal models for translational research in cartilage repair. *J. Exp. Ortop.* **2016**, *3*, 1–12. [CrossRef]
52. Seaton, M.; Hocking, A.; Gibran, N.S. Porcine Models of Cutaneous Wound Healing. *ILAR J.* **2015**, *56*, 127–138. [CrossRef]