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Chapter 11

Designer Supersurfaces via Bioinspiration and Biomimetics for Dental Materials and Structures

David W. Green and Han-Sung Jung

Abstract The design of surfaces and interfaces gives rise to superior qualities and properties to materials and structures. The interface between biology and materials in nature is being closely examined at the smallest scales for a number of significant reasons. It is recognised that the properties of surfaces have definite biological effects that can be harnessed in clinical regeneration biology. Also the deeper understanding of surface interactions between cells and matrices in human biology is spurring the fabrication of biomimetic and bioinspired versions of these natural designs. The new emerging science of bioinspired surface engineering is helping to improve clinical performances for biomaterials and biostructures because it resolves the problems necessary to optimise integration of implant biomaterials and structures. One of the major developments is the use of surface topography, which is now being exploited for microbial control, steering stem cell behaviours in proliferation and differentiation and adhesive surfaces for better bonding with tissues. In this Chapter we will explore the status of these super surfaces and examine the possibilities for the next generation of dental biomaterials and implants.

Keywords Antibacterial surfaces • Bactericidal surfaces • Cell influencing surfaces • Microtopography • Surface nanotopography

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11.1 Introduction

The structural and chemical details at surfaces of biomaterials and the meeting between surfaces is vitally important in the mechanical design of organisms, structural biomaterials, anti-wetting, self-cleaning properties, cell adhesion and migration. These superior and sophisticated properties are what can be termed super surfaces. Evolution has selected for adaptations that include various styles of physical structuring, chemical coatings and molecular patterning to create superior and sophisticated functions at surfaces. These are the best possible adaptations, in the design of surfaces that also apply to the same intrinsic problems faced in applications for biology and medicine. They have been tried, tested and optimized over millions of years of evolution. A result is that many of the adaptations discovered in nature are often new to science and technology. Hence this is the reason why biomimetic based researchers search across nature for new potent ideas in solving materials based problems. There are added advantages in following biomimetic approaches such as, learning how to reduce energy during the construction of materials and features at the surfaces [1]. There are now large catalogues where this kind of innovation information can be easily accessed, interpreted and used for the interrogators problem in hand [2]. An important distinction is to be made between biomimetic and bioinspired approaches. In biomimetics the objective is to simulate or copy a structure, process or mechanism directly from nature. Bioinspiration is the strategy where an influential component from biology is used in the problem solving and its eventual solution. So with bioinspiration there is a confluence of biological and human ingenuity. Each strategy has been used effectively in biomedicine.

In this chapter, we focus on two biomedically significant topics where the design of surfaces can be improved for better clinical outcomes. These topics are bacterial and human cell adhesion and detachment. Specifically, the clinical problem at biomaterial implant surfaces is to drive a strong yet stable biointegration and the second is an effective control of pathogenic microbes at the outer surface of implants. The construction and refinement by optimisation of the surfaces and interfaces of traditional restoration dental materials is a large topic of research but will not be included here. Material scientists are infact still grappling to control these phenomena and having the ability to programme their surfaces to work in tune with biology. The examples we will focus on in this chapter for developing biomedical supersurfaces are mainly studies in bioinspiration.

A major quest for regeneration scientists is the ability to control cell behaviour and activity for a variety of roles. Cell manipulation engineers have achieved some success in defining the mechanisms for influencing cells in predictable ways. Cells are influenced and guided by physical forces and contacts with surfaces. This environment conditions the cells future role. This means that cells in tissue organizing collectives are ultimately programmed outside in than inside out. Considerable research has been underway to develop surface features that can be used to sensitize and direct cell growth, proliferation and differentiation. More advanced

surface engineering employs changes in the characteristics of topography, symmetry, geometry, stiffness and elasticity of the underlying material all-together. It has been challenging to systematize all of these elements into cause and effect relationships. The desire is to produce a blueprint for designs that have predicted effects. Programmable biomaterials with influential topography are a realistic prospect for interplay with human cells and bacteria cells. There is tremendous array of data showing the diverse pairings of nanotopography arrays with fibroblasts, endothelial, epithelial, pluripotent, mesenchymal and embryonic [3–12]. There are numerous instances of conflicting results but there are strong trends emerging. For example, certain topographic structures induce clear differentiation responses within contacting cells. The best example is osteogenesis by Mesenchymal Stem Cells (MSCs) subject to disordered nanopits [13]. Significantly adding to this is evidence of the molecular pathways involved in this process, the main one being integrin-activated focal adhesion kinase (FAK). Another trend is that low aspect ratio structures are favourable to attachment and spreading phenomena whereas higher aspect ratio structures lead to cell sheets that self detach [14].

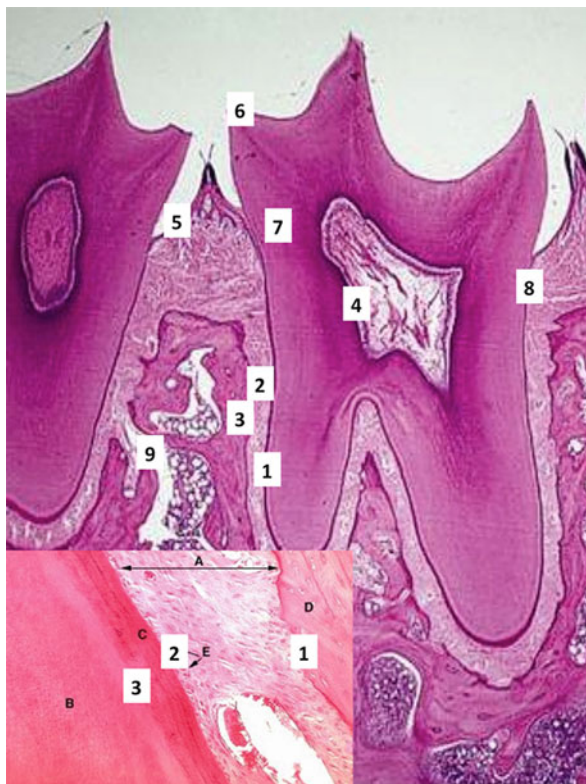
Eukaryotic and Prokaryotic cells are also influenced strongly by the chemistry of the surface. The chemistry aspect and the physical features are interlinked. Each influences the downstream effects of the other factor. A surface with a homogenous chemistry on a smooth surface once modified with surface topography redistributes the chemistry and introduces new heterogeneity. In the next section, we map the surfaces and boundaries in and around the tooth organ and describe briefly their biological and mechanical functions.

11.2 Materials Dentistry: A World of Surfaces and Interfaces

Restoration and replacement of dental structures is intensively focused on surfaces at boundaries and interfaces. The tooth organ is made up of a multiplicity of tissue layers and interfaces (Fig. 11.1). These are necessary for the intricate biomechanical functions of the tooth organ. Replacing them requires mastery of interface engineering. Graded interface is the key to integration between layers consisting of different compositions and structures. Many mechanisms are in play to stop or contain cracks from forming. In traditional restorative dentistry the question of bonding layers of different materials coherently has been studied in great depth. The better design of surface structures and chemistry is imperative for every material placed inside the body. Surfaces are also being used to control and manipulate biology in rational ways.

In the regenerative sciences precise control of cell proliferation and differentiation is unresolved and therefore remains of considerable future significance. In cell engineering surface structures over large surface areas have been developed to select, maintain, expand and invoke phenotype changes in cell populations with

Fig. 11.1 A histological longitudinal slice through a human molar tooth with annotations to highlight surfaces and junctions or interfaces inside and around the tooth organ. (1) Dentine to Periodontal ligament interface; (2) PDL/cementum interface; (3) Cementum/bone junction; (4) Dentine/pulp junction; (5) Gingiva boundary; (6) Enamel boundary; (7) Dentine/enamel junction; (8) Gingiva/enamel interface. Image reproduced from: <http://www.uky.edu/~brmacp/oralhist/module8/lab/imgshtml/image02.htm> and <http://www.am-medicine.com/2013/12/an-illustrative-note-powerpoint.html>



some important successes. Topography at the nanoscale is showing enormous promise as a device to influence cell behaviours in predictable and useful ways for benefits in cell therapy and tissue engineering. Research on surface continues to be a crux in materials dentistry and regenerative dentistry. The major areas would be surfaces for bacteria control and selectivity and surfaces for cell and tissue integration. The basic work on programmed surfaces for cell selection, growth and lineage specification also relate heavily to regenerative dentistry strategies and offer new therapeutic routes. In the next sections of this chapter we hone in on the programmed surfaces with topography for bacteria control, tissue adhesion and biointegration.

11.3 Bactericidal and Antibacterial Surfaces

Bacterial biofilms are notoriously difficult to eradicate from surfaces such as implants. There are different ways of preventing bacteria adhesion and colonisation. The first most extensively investigated is chemical and molecular engineering of surfaces. In these approaches surfaces are built with adjuncts such as dendrimers,

cationic peptides, photoactivation, lysostaphin, deactivators of quorum sensing and grafted antibiotics [15].

In dentistry, there is the added complexity by which the main aim is to selectively control different bacterial populations and not to eradicate everything. The mechanisms of attachment for bacteria are not fully understood. Surface roughness, wettability and surface energy are known to influence bacteria attachment and adhesion most profoundly. The range of limits for these properties has been difficult to measure precisely. Surface roughness above $0.2\ \mu\text{m}$ is known to promote plaque formation. The influence of surface energy properties is complicated by the nature of the bacterial cell wall charge properties. Hydrophobic interactions in bacteria are common since adhesions located on pilli are themselves hydrophobic. According to some evidence acquired in vitro hydrophobic processes drives attachment. However, the greatest task is to unravel the complexities of surface properties and bacteria adhesion in living biological environments. Of greatest prominence is the effect of serum proteins at the surface, which conditions all other biological responses.

11.3.1 Controlling Oral Pathogens via Surface Structuring

The oral cavity is colonised by a whole community of microbes that include bacteria, viruses and fungi. The ecology or interrelationships between the members of the various microbial communities are highly intricate and under constant investigation with new links in the network being uncovered regularly. It is thought that changes in community structure invoke degenerative diseases that cause tissue destruction of dentine, periodontal ligament, gingiva and bone. Once the environment and conditions favour the acceleration of pathogenic growth the disease and tissue destruction is highly likely to occur. Effective ways must be sought to control and eradicate pathogenic microbes from the mouth. A degree of control is often required to reset the community structure of bacteria. There has been voluminous research to effectively kill pathogenic outright. Antibiotics are the most effective altogether. However, there is increasing evolved resistance to antibiotics and the targeted delivery of antibiotics remains imperfect. Other main treatments implement chemical toxins, photodynamic elements and nanoparticles to destroy bacterial biofilms and kill bacteria. There is also renewed interest in prospecting for new antibacterial compounds from sessile invertebrates renowned for the complex defensive chemistry, e.g. Marine sponges and Ascidians. As such there are many examples in nature where evolution has selected for sophisticated adaptations to kill microbes or prevent contact with the organism. A significant adaptation that has emerged is structural devices at surfaces.

Nature has evolved countless interfaces precisely with anti-bacterial defences using specific Nano topographies alone. And this is independent of the effects from chemical secretions. Probably the first application of patterned surfaces of diamond shaped micro-protuberances to hinder bacterial contamination is Sharklet inspired

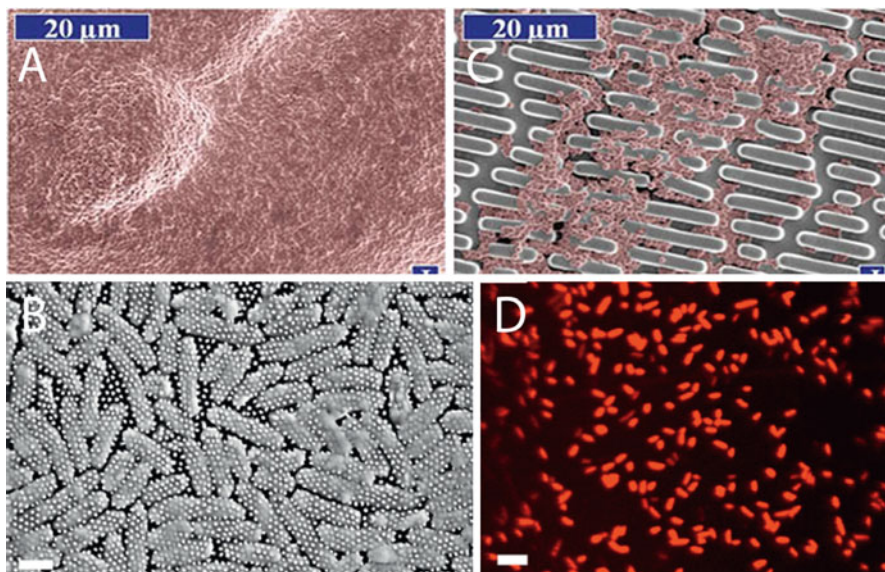


Fig. 11.2 Anti-bacterial and Bactericidal surfaces based on microstructure and nanostructure. (a) Smooth surface covered in bacteria after 2 days; (b) Bacteria colonisation on a patterned microstructure surface translated from shark skin; (c) SEM of “skewered” bacteria; (d) Confocal image showing dead bacteria sitting on top of Cicada nanopillar structure [18]

from the micron structure of scales or dermal denticles from shark-skin [16, 17]. The synthetically replicated surface hinders growth of a range of biomedically significant bacteria species such as, *Staphylococcus aureus* and *Escherichia coli* [16, 17]. The special nanostructure at the surface are deleterious to *Pseudomonas aeruginosa* and lead to the shredding of other pathogenic species including: *B. catarrhalis*, *E. coli*, *P. aeruginosa*, and *P. fluorescens*. Another recently discovered bactericidal surface imported directly from nature is the Cicada wing surface (Fig. 11.2).

The structure consists of nanometric pillars 200 nm tall, 100 nm in diameter at the base and 60 nm at the tip spaced 170 nm apart in a highly regular and tight pattern. This precision piece of Nano architecture being ten times smaller than the cell itself punctured settling bacterial cells and killed them with 60 min from attachment (Fig. 11.2). The killing power has been measured for this wing surface and was described as being efficient with 6×10^6 bacterial cells made inoperable in every square centimeter after 30 min [18]. These initial results represent are of supreme usefulness for control of clinical infections anchored onto biomaterial and implant surfaces. However, the topography did not kill gram-positive species of bacteria: *B. subtilis*, *P. maritimus*, and *S. aureus* species of bacteria. Other wing topographies are being actively pursued as potential antibacterial and bactericidal devices. It has been reported that Dragonfly wings *Diplacodes bipunctata* have strong and rapid bactericidal effects on a broader range of bacteria classes-both

gram negative and gram-positive types as well as bacterial spores. A synthetically created surface with the exact same features of densely packed protruding nanopikes as the Dragonfly wing demonstrated the same bactericidal effects. It was estimated that 45,000 bacterial cells every minute in every cm squared were killed. Black silicon is this equivalent and is generated using ion beam technology. This is costly and cannot be transferred onto just any surface and specifically onto the type of materials useful in biomedicine [19].

Surface roughness and structure influences human cells more acutely than bacterial cells. This is because eukaryotic cells have a much more complicated sensory apparatus than prokaryotes. It was first evidenced that human cells can sense, detect and “react” to structures of >5 nm at very small distances of 3–15 nm [20]. Physical attachments between cells and extracellular matrix (ECM) molecules can only be made at such close distance. There is broad remit to harness the sensory apparatus of the cell and influence their behaviour in many important aspects such as, migration, alignment, polarity, differentiation and proliferation. Such governability opens up many biotechnological and therapeutic avenues from tissue regeneration to biosensing.

11.4 Cell Adhesive Surfaces Using Nanotopography

Material surfaces with higher and more potent capacities to encourage cell attachment are required in a range of biomedical applications. This is achieved by modulating the type of nanostructure and its dimensions. Nanopillars have recently shown a degree of success in selectively adhering cells onto its structure with clear effects on phenotype and proliferation (Fig. 11.3a, b). In many applications adhesion and separation of different cell types is a desirable biological event (Fig. 11.3c). For example in one study nanopillar structures of a specified aspect ratio would favour endothelial cell adhesion while concomitantly preventing adhesion of fibroblastic cells. This duality is ideal for vascular implants in, which endothelial association is needed for coating and the fusion with existing vessels without interference of fibroblasts involved in clotting reactions [4] (Fig. 11.3c). Strong cell attachment on specialised cell adhesive nanotopographies is a vital outcome that can promote tissue formation, remodelling and bonding at the bio-material surface.

11.5 Tissue Adhesive Surfaces

Materials with surfaces that can adhere to living tissue and participate in regeneration, development and repair are important. In surgery tough, stretchable and tear resistant tapes able to stick rigidly to tissues would be broadly revolutionary in the treatment of wounds, reducing surgery and complications. Conceivably such a

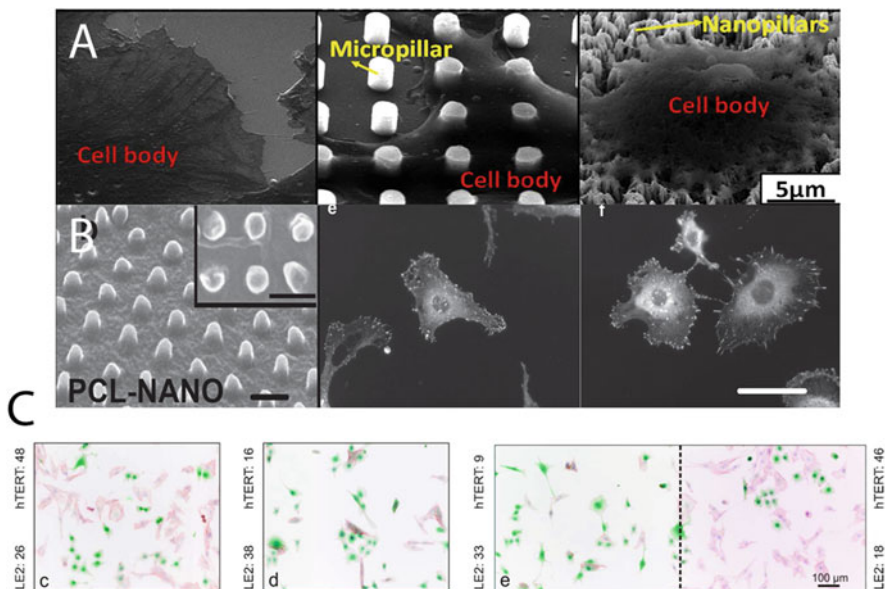


Fig. 11.3 Cell responses to nanopillar topography. Variations are apparent in responses regarding different cell types and dimensions of the nanotopography. (a) MSCs growing on top of nanopillars did not spread and the shape governing stress distribution increased production of osteogenic matrix molecules; (b) Vinculin staining to highlight the focal adhesion portion inside hTERT (fibroblasts) cells at cell periphery on flat and nanopillar array. The low count of focal adhesions on nanopillars decreased proliferation; (c) Co-staining of hTERT and endothelial cells to show the different growth and proliferation responses with increasing pillar size (aspect ratio) from left to right. Endothelial cell growth and proliferation were preferentially selected on the high aspect ratio nanopillar surfaces [4, 5]

design could be used to replace sutures and staples. Bioglues have been developed as potential candidates for wound closure and sealing. However, they have been dogged by inflammation susceptibility. The reason is that the toughening of these tissue adhesives requires strong chemical reactions to take place, and is the source of biological irritation. Another point is to develop effective glues that bond in wet conditions. In both cases natural ingenuity may offer prospects for success. Adhesives derived from nature may offer a chemistry of bonding which is more favourable to biological systems and less inflammatory. In this vein, analogues (e.g. polydopamine) of the main active ingredient of mussel adhesive proteins, 3,4-dihydroxyphenylalanine (DOPA) have been broadly investigated.

The topic of bioadhesives is large and is focused upon chemical compounds assimilated with potent chemical reactions—a necessity in making tough and resilient materials for the task. This has the unintended consequence of eliciting inflammation. In a bioinspired approach the idea has been to harness naturally occurring surface structures for adhesion such as the Gecko foot pads; reproducing them in a biocompatible elastomer in the role of a self-adhesive tissue tape (Fig. 11.4a). Along these lines it was proposed that adhesion is largely based on

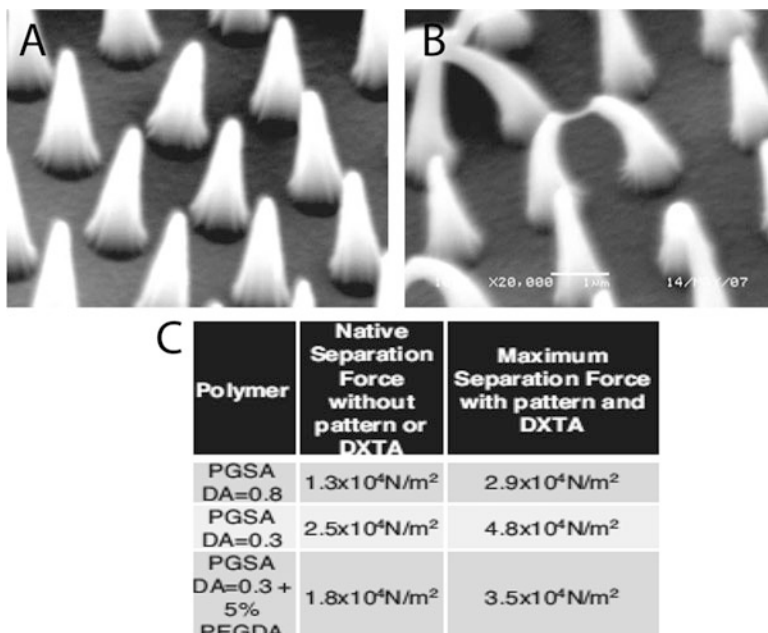


Fig. 11.4 Nanotopography copied from the design of Gecko footpad setae were used to produce an self-adhesive tape material. (a) High power SEM of nanoprotusions made from elastomer; (b) High power SEM of nanoprotusions coated with a dextran coating to enhance tissue adhesion; (c) Table showing the strength of attachment to porcine intestine tissue between non nanotextured and textured with and without oxidised dextran [22]

physical structure. The problem of wet adhesion has been solved in nature by Tree frogs for example. The design blueprint has been unravelled in this organism and is therefore accessible for technology transfer into a useful product [21]. In the Gecko example wet adhesion property had to be introduced by additional chemical coating (Fig. 11.4b). The bioinspired engineers developed a strongly adherent tissue tape copied from the structure design of gecko foot pad surfaces. In tests the tape performed well on porcine intestine tissue and rat abdominal subfascial in vivo with strong forces of resistance to its separation from the living wet tissue (Fig. 11.4c) [22].

11.6 Surfaces for Cell Proliferation and Differentiation

Structures at surfaces that elicit proliferation and/or differentiation responses are in high demand especially those with high potency and precise reactions [11, 12]. A principal property of the surface with biological implications is wettability feature [23]. Still more information is needed to completely understand the effects of wettability on cell attachment and tissue integration. Surprisingly for dental

implants the wettability is usually not measured or considered in biological evaluation. The topic has been scrutinised most widely for implant osseointegration [23]. Generally wettabilities of intermediate values can optimise favourable cell interactions. An important contribution of wettability to biodynamics at the surface is protein adsorption. Proteins are the first biomolecule to arrive at the surface taking milliseconds. The nature of the protein assembly at the surface directs the cell response. This has been studied mainly with osteoblasts as well as fibroblasts and keratinocytes. Synergism between topography and chemical properties occurs but the interrelationship is unpredictable.

One of the purposes is to discover and develop the most efficient platform of expanding the numbers of stem cells *in vitro* into the population numbers needed for therapeutic tissue regeneration. In addition the ability to specify cell lineages of the expanded populations is another necessity to generate desired tissue types. Once again platform cell-scale microgauged technologies that can achieve this accurately and with high specificity are still needed. These base technologies are useful for the study of basic processes and in modelling responses to new drugs and to build phenotypically accurate populations of cells for tissue regeneration. Much work has been carried out to unravel the mechanisms involved in surface contact and gene expression. The principal contact point is the subcellular macromolecular focal adhesion, which is joined between the cell cytoskeleton and extracellular matrices [7]. The association and clustering of these objects with the matrix is an important effect that allows sensing of mechanical forces. Others have discovered the molecular circuits directly involved in transmitting topography influences into the cell nucleus where it impinges on gene expression patterns.

To be truly biomimetic with the totality of biological functions the surface patterns and design should ideally relate directly to the surfaces of the extracellular matrix and structural biomaterials (Fig. 11.5). Cell function is strongly influenced by active structures at nanometric sizes. Going beyond this, introduced nanoobjects such as rods, particles and fibres interplay with cells at the nanoscale by influencing extracellular micro-physiological events including protein adsorption and receptor-ligand binding. In one good example the ultrastructure details of the extracellular matrices were used to plan the design of synthetic topographies [24]. Thus this strategy has a strong biological basis to it. Many ECM structures possess nanogauged groove structures for example. In this study the researchers used the structural density of nanofeatures whose features were replicated from model tissues bone, nerve and skin. They found that processes such as adhesion, migration and differentiation could be controlled directly via the spacing and density of nanotopographic grooves [24]. The geometries of natural surface structures are being increasingly assessed as potential platforms for MSC differentiation and maintenance [13]. In nature the geometrical patterns are often more intricate than regular grooves, pillars or pits [25]. In the first report of its kind disordered arrangement of nanopits was found to stimulate osteogenic differentiation of MSC's. Recent similarity has been drawn between this geometry for MSC's and the nanofeature of collagen X on endochondral ossification because it shows a hexagonal pattern (Fig. 11.5) [26].

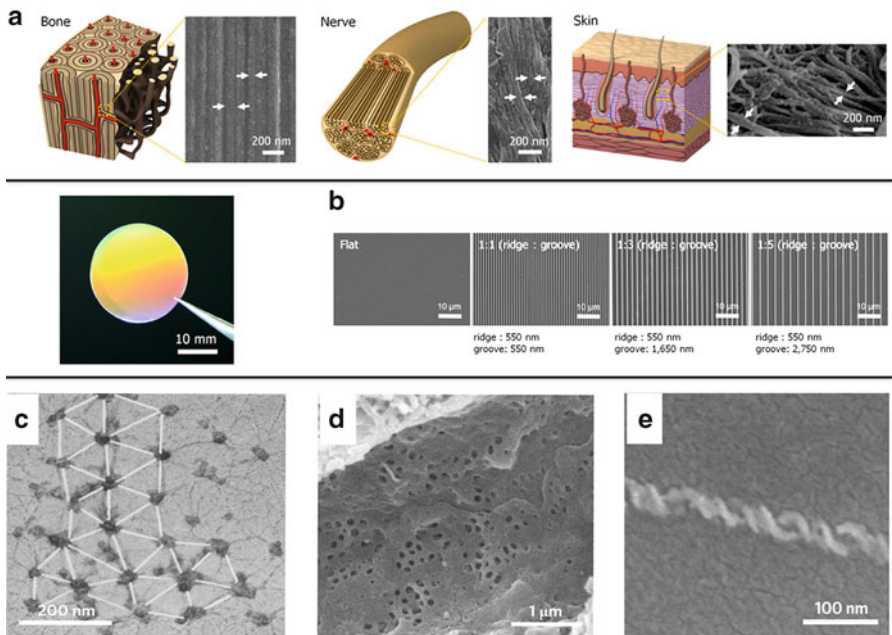


Fig. 11.5 Selection of biological models for nanotopography in new synthetic materials. (a) Natural ECMs of bone, nerve and skin possess regular nanogroove architectures as shown in the SEM images [24]; (b) In this highlighted study nanogrooves with similar dimensions to groove structures in native tissues were printed onto artificial surfaces and tested for stem cell responses [24]; (c–e) Native 2D surface environments are often disordered and not regular, or show chiral patterns. (c) Periodicity in Type X collagen [26]; (d) Sinusoidal capillary with disordered pore arrangement [13]; (e) An artificial helical fibre with the same 63 nm periodicity of natural collagen, which was discovered to induce osteogenesis in mesenchymal stem cells

11.7 Conclusion

The interplay between cells and surfaces directs the future activity and behaviour of the contacting cell population. This interaction can be designed or programmed by physical and chemical patterning using sophisticated machines. Originally the patterning geometries did not have equivalents in biological systems. Increasingly cell engineering via surfaces is being led by mimicking the patterned features on ECM supramolecules and other structures. The physical characteristics used to influence cells on contact include: topography, stiffness and elasticity. A lot of promising results have emerged through the different shaping of nanotopography, which cells can sense. We interpret this sensing feature to result from adaptations to sense features of extracellular matrices that are constructed from nanogauge objects and display nanofeatures in the final ECM product. We highlighted how nanotopography is helping to control bacteria populations and to stimulate stem and pluripotent cells into deliberate actions using natural Cicada wing structures. Construction of a systematic order is needed to connect a feature by shape or

dimension with a single or collective response by a cell. We also highlighted the utility of topography design on the physical attachment and biointegration with different tissues. In one instance a group of bioengineers successfully demonstrated the strong tissue attachment of a polymer membrane patterned with nanopillars, and augmented with oxidised dextran, but inspired from the structure and adhesive properties of small hairs on the Gecko footpad. Thus, bioinspiration methodology could be the guide for the next design of plaster for wound healing inside the oral cavity. Biomimetic and bioinspired nanotopographies mined from nature are largely unexplored in these areas of dentistry.

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