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MOLECULAR BIOLOGY OF SKELETAL TISSUE ENGINEERING (DW HUTMACHER, SECTION EDITOR)

New Bioinspired Materials for Regenerative Medicine

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Abstract Bone and cartilage regeneration is an important part of tissue regeneration where bioinspired materials have a great impact. The review provides an overview of the biology of bone and cartilage regeneration, the cells used, as well as the materials and systems used in the field, closed by a section of interesting cofactors and sterilization methods. First, an overview of the biology of bone and cartilage regeneration is presented in combination with the corresponding cells most often used in bone regeneration as well as important factors involved in bone and cartilage regeneration. In the second section, some fundamental aspects of bone and cartilage will be briefly introduced. In the third section, new developments in bioinspired materials will be highlighted, ordered by the class of material: bioglass, hydroxyapatite, and natural and synthetic polymers. In the fourth section, new concepts for material modification are introduced: the use of nanotechnology, supplementing factors, and the impact of sterilization.

Keywords Bone regeneration · Cartilage regeneration · Bioglass · Nanotechnology

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Introduction

Skeletal tissue consists of craniofacial, mandible, cartilage, ligament/tendons, and most predominately bone. In this review, some of the new findings concerning the use of bioinspired materials in cartilage and bone regeneration will be reviewed, with emphasis to bone regeneration.

The review briefly summarizes bone and cartilage regeneration and reviews new developments in bioinspired materials for regenerative medicine. After a short introduction of the cells applicable for bone and cartilage regeneration, the tissues cartilage and bone are introduced. The next section focuses on new material developments in tissue regeneration. These materials span a wide range from bioglass to natural and synthetic polymers. Due its dominant role, a major emphasis will be on bioglass. Bioglass is a calcium silica glass which contains sodium and phosphate [1]. Hydroxyapatite is often used as scaffold material or as supplement in other scaffold materials [2] for bone and cartilage regeneration.

Several cells are applicable in both bone regeneration and cartilage regeneration (for details, see Table 1). The most important cells are mesenchymal stem cells (MSCs) [16]. As alternative, dental pulp stem cells, which can also differentiate into osteoblasts and chondrocytes, are considered as potential candidates [6, 5, 17•] (Table 1).

Table 1 Cells useable for cartilage and bone regeneration and cells useable for bone regeneration

Abbreviation	Name or description	Application	Literature
MSC	Mesenchymal stem cells	Cartilage	[3]
MSC	Mesenchymal stem cells	Bone	[4]
DPSC	Dental pulp stem cells	Cartilage	[5]
DPSC	Dental pulp stem cells	Bone	[6]
MG63	Human osteosarcoma cell line	Bone	[7–9]
Saos-2	Human osteosarcoma cell line	Bone	[10]
MC3T3-E1	Murine (mouse)-derived osteoblast cell	Bone	[11–13]
7F2	Mouse osteoblast cell	Bone	[14]
UMR-106	Rat osteoblast cell	Bone	[15]

Beside these cell lines, several cell lines are only useable for bone regeneration. The most often used cell line in this context is osteoblast-like cells (osteosarcoma cell line) MG-63; some more cell lines are also mentioned in Table 1.

The most important cells for cartilage repair are MSC and chondrocytes. The mesenchymal stem cells can have different origins like bone, adipose, synovial, or, more seldom, periosteum, trabecular bone, and umbilical cord blood [18]. Quite often, articular chondrocytes are used, an interesting alternative are nasal chondrocytes [19•]. Cartilage progenitor cells could also be a possible cell source [20•, 21], as well as chondrosarcoma cell line SW-1353 [22]. An overview over different cells useable for cartilage repair was recently reviewed [23•].

Recent advancements in tissue engineering mimic the complexity of biological tissues by applying cell co-cultures as more biorelevant systems. Some co-cultures are examined for example in order to reduce the amount of chondrocytes needed by co-culturing them with MSC; a culture of 1:1 chondrocytes to MSCs showed positive results [24]. Chondrocytes were co-cultured with adipose-derived mesenchymal stem cells allowing to reduce the damage of chondrocytes by oxidative stress; the co-cultured cells were less susceptible towards 200 μM hydrogen peroxide and expressed more collagen-II-α [25].

Some factors important for the differentiation towards the osteogenic or chondrogenic cell type and factors specific for the right cell type are shown in Table 2 and in the cited literature (Table 2).

The differentiation of MSCs towards osteoblasts is mainly regulated by the genes RunX and Osterix (OTX). The following bone-related genes are expressed: alkaline-phosphatase (ALP), bone sialoprotein (BSP), type 1 collagen (Col1), osteopontin (OPN), osteonectin (SPARC), and osteocalcin (OCN) [13].

In case of chondrogenic differentiation, SOX9 triggers the expression of aggrecan and collagen type II, which are important for differentiation [27••].

Components of Skeletal Tissue

Cartilage

Cartilage consists of four different zones: superficial, transitional, middle (radial), and calcified cartilage zone. They differ in cell density, collagen, and proteoglycan amount [28, 29, 30]. The constituting collagen is mainly collagen II (90–95 %), but also some other collagen types, e.g., collagen VI, IX, X, and XI, are found [29].

In the superficial zone, the amount of collagen is high and the chondrocytes are flattened; additionally, there is less proteoglycan compared with other zones [29]. In the transitional zone, the chondrocytes have a spheroidal shape, the cell density is lower, and the collagen fibers are randomly aligned [28, 29]. The cells in the middle zone are round and have high synthetic activity, and the collagen fibers are perpendicularly aligned towards the joint surface [29]. In the calcified zone, the chondrocytes are smaller and some have little metabolic activity [29]. Collagen II fibers are present in all zones of cartilage, in the calcified zone additionally collagen X [30]. A schematic of the cartilage structure can be seen in Fig. 1.

An overview about cell therapies in cartilage regeneration can be found in current reviews [31, 32].

Bone

Bone is a complicated structure reviewed in several reviews [33, 34•, 35]. On the macroscale, one can distinguish two types of bone: cortical (compact) bone and trabecular (cancellous, spongy) bone. Cancellous bone is metabolically more active than cortical bone and is remodeled more often [33]. There two kinds of cortical bone, woven and lamellar bone, which are distinguished in the way the collagen fibers are arranged. In the woven bone, no organization of the collagen fibers can be visualized [33]. As woven bone is formed quite rapidly, it is formed first after fracture and is then remodeled

Table 2 Factors in skeletal regeneration [13, 16, 26, 27••]

Abbreviation	Name	Kind	Stage
Runx2	Runt-related transcription factor 2	Osteogenic	Osteogenic differentiation
OTX	Osterix	Osteogenic	Osteogenic differentiation
ALP	Alkaline phosphatase	Osteogenic	Bone matrix formation
COL1	Type 1 collagen	Osteogenic	Bone matrix formation
BSP	Bone sialoprotein	Osteogenic	Bone matrix formation
OPN	Osteopontin	Osteogenic	Bone matrix formation
OCN	Osteonectin	Osteogenic	Bone matrix formation
SPARC	Osteocalcin	Osteogenic	Bone matrix formation
SOX9	SRY-box containing gene 9	Chondrogenic	Early-stage chondrogenesis
GAG	Glycosaminoglycan	Chondrogenic	Chondrogenesis
COL2	Type II collagen	Chondrogenic	Maturation of cartilage
COlX	Collagen X	Chondrogenic	Maturation of cartilage
Aggrecan		Chondrogenic	Maturation of cartilage
COMP	Cartilage oligomeric matrix protein	Chondrogenic	Maturation of cartilage

into lamellar bone [34•, 36]. In lamellar bone, the mineralized collagen fibers are ordered in lamellae which contain ordered and disordered material with embedded canaliculi [34•]. For more information about the structure of bone, e.g., lamellar bone, the reader is referred to recent reviews [34•, 35].

Bone consists of organic phases, minerals, and water. The organic phases mainly consist of collagen I (90 %), some other types of collagen, and 10 % non-collagenous proteins [35]. The minerals are carbonated hydroxyapatite which assembles within the gap of the collagen fibrils. They have a plate-like structure with some tens of nanometers in length but only 1–2 nm in height [34•, 37].

The fracture toughness differs with the type of bone and the age of the person; typically, 3–10 MPa are found [35, 37]. The elastic modulus from bone spans from 15 to 25 GPa [37].

Structures for Tissue Engineering

Bioglass

Bioglass is an often used glass ceramic in bone regeneration. The most important bioglass is 45S5, which has the composition 45 % SiO_2, 24.5 % Na_2O, 24.5 % CaO, and 6 % P_2O_5. This composition is close to a ternary eutectic phase and hence easily meltable. Bioglass was invented by L. Hench [1]. The amount of the different compounds is important for biocompatibility; compositions with more than 60 % SiO_2 are bioinert [1].

Normally, high temperature is needed to produce bioglass by sintering. Some attempts to produce bioglass at room temperature were made, for example, by using calf thymus DNA

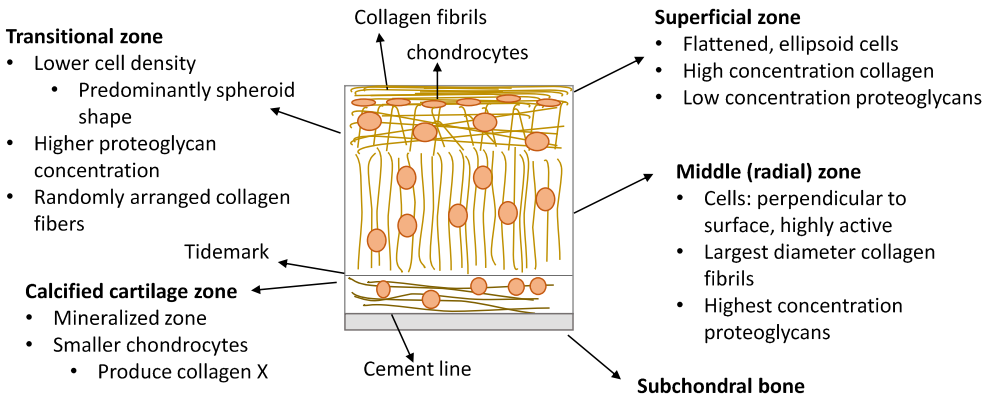

Fig. 1 Schematic representation of the different cartilage zones. The superficial zone consists of two layers, one acellular sheet of mainly collagen, beneath a layer where the chondrocyte lay flattened parallel to the articular surface [29]. In this zone, collagen prevails and only a low amount of proteoglycan is found [29]. In the transitional zone, the cell density is lower, while the amount of proteoglycan is higher; the collagen fibers are randomly aligned [28]. The middle (radial) zone is marked by the lowest amount of cells, which are oriented perpendicular to the surface and have high metabolic activity [28, 29]. The radial zone and calcified zone are separated from the radial zone by the tidemark, a basophilic line [29]. In the calcified zone, the chondrocytes are smaller and produce not only collagen II but also collagen X [28, 29]

as a template, which was used successfully to produce bioglass at an ambient condition and tested with osteosarcoma cells [38].

Bioglass has been included into other matrices like polycaprolactone, chitosan, polyvinyl alcohol/chitosan collagen hybrids, and hydroxyapatite [10, 11, 15, 39]. The addition of bioglass led to better mechanical properties and normally better biocompatibility of the scaffolds. Bioglass, bioglass-hydroxyapatite, and mineral trioxide aggregate were compared towards their biocompatibility to Saos-2 cells, with the result that hydroxyapatite-bioglass was most biocompatible [10].

Different ions were added to bioglass to enhance biological properties, like strontium [11], fluoride [40], strontium hexaferrite [41], boron [42, 43], zinc [44, 45] and europium [46], gallium [47•, 48], titanium [49, 50], copper [50, 51•], manganese [52•], and silver [52•]. An overview over the different elements in the body, their function, and their application in bioactive glass is provided in the review of G. Kaur [53•].

Fluoride was reported to facilitate the formation of apatite with higher crystallinity at pH below 6, as long as the fluoride content is not too high [40]. Strontium hexaferrite nanoparticles were added to generate a material for hyperthermia treatment of bone cancer, but the biocompatibility is reduced by the addition of strontium hexaferrite [41]. Boron as dopant showed enhanced vascularization [42]. 45S5 bioglass doped with 2 % B_2O_3 was tested in vivo with the vasculature of the chorioallantoic membrane of an embryonic quail, and stimulated angiogenesis could be shown [43]. Doping bioglass with 5 % zinc leads to apatite formation in simulated body fluid (SBF); in case of doping with 10 % zinc calcite is produced [45]. Europium was introduced to produce luminescent bioglasses with an intended application in drug delivery. The physicochemical properties and cell viability were tested as well for this system. The cell compatibility of the europium bioglass was shown to be dependent on the europium concentration [46]. Including 4 % of titanium into bioglass supports cell viability and apatite formation but decreases cell adhesion [49]. Copper and titania have an impact towards the thermal properties of the bioactive glasses, copper decreases the glass transition and crystallization temperature, whereas titanium increases them [50]. Manganese retards the precipitation of hydroxyapatite (HA) but has no cytotoxic effects, and it enhances expression of ALP and bone morphogenetic proteins (BMP) [52•]. Silver and titanium lead to a decrease of the melting temperature; towards the glass transition temperature, silver has no effect [54].

To improve the mechanical properties of bioglass, several coatings were used: nanocellulose [7], poly-DL-lactide [55], poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) [56], and polyvinyl alcohol (PVA) as well as microfibrillated cellulose (MFC) [57]. The cells proliferate better on poly-DL-lactide-coated bioglass than on pure bioglass [55]. Coating with PHBV led to better mechanical properties and more controlled release of vancomycin compared to direct loading of the drug in the bioglass [56]. Bioglass scaffolds were produced from an ethanol-based slurry and coated with PVA or PVA with MFC; the coating with PVA led to a fivefold increase of compressive strength, and coatings with PVA/MFC led to a tenfold increase of compressive strength [57]. Five coatings of bioglass were compared concerning their influence on bioglass stiffness. Polycaprolactone and collagen coatings increased the stiffness ultrasonically measured, while neither alginate nor poly-L-lactide nor gelatine coating increased the stiffness [58]. In another study, coatings of bioglass were compared for their impact towards stiffness: PHBV, gelatine, cross-linked gelatine, alginate, and cross-linked alginate. The coatings led to higher stiffnesses of the 45S5 bioglass and which was further increased by cross-linking the natural polymers [59]. Comparing the results of the two studies indicates that 1-min immersion in an alginate solution is too low to improve the stiffness [58], while two times of 5-min soaking improves the stiffness [59]. The reason that gelatine could improve the stiffness in the study [59], but not in the other, could be the different solution temperature of 60 °C in the first [58] and 50 °C [59] in the second experiment, or the concentration limit for gelatine in bioglass lies under 8 wt% [58], approximately around 5 % [59]. Thus, comparing different weight percents of gelatine for coatings with different solution temperatures would be important to be investigated in the future.

Hydroxyapatite

HA is the main mineral component of bone. Hence, it can be regarded as an appropriate matrix for bone regeneration [35].

It was shown that cell seeding of MSC in hydroxyapatite scaffolds could not improve bone healing, compared to non-cell-seeded hydroxyapatite scaffolds [60]. Hydroxyapatite scaffolds with a pore size of 800 μm were synthesized by selective laser sintering (SLS) in a two-step sintering approach; in SBF, a bone apatite-like layer was formed and the cell compatibility was tested with MG63 osteoblast cells [61]. Composite scaffolds were formed with several polymers like polyamide 66 (PA66) [62•], poly-caprolactone [63], polylactide, collagen [64, 65], and chitosan [66] or with proteins like amyloid [67].

In case of a PA66, a composite of 40 % HA/PA66 prepared with a shot size of 23–25 mm by injection molding showed the best properties concerning pore size (100–500 μm) and good mechanical properties [62•]. Scaffolds produced by fused deposition modeling with a composition of PCL:HA (60:40) showed new bone formation after 12 weeks in vivo; incorporation of BMSCs increases bone healing [63]. The viability

of MG63 osteoblast cells in collagen/HA composite scaffolds was increased with increasing amount of HA; for the investigated scaffolds, collagen:HA 30:70 was the best weight ratio [65]. A chitosan/HA hybrid scaffold with 10 wt% HA was produced by in situ hybridization and lyophilization; the mechanical properties matched cancellous bone and the pore size of the channel pores was 150–650 μm. Adding RGD to the scaffold led to better attachment of the MSCs and higher ALP activity [66]. In some studies, modification of the HA or the matrices was used to influence the structure of the scaffold. Arginine incorporation facilitated the formation of homogenous nanoplate-like HAp in collagen or chitosan in a bioinspired sol-gel process at 400 °C; samples without arginine produced brushite or monite crystals [68•].

Natural Polymers

Natural polymers for bone or cartilage regeneration mainly used are chitosan, collagen, hyaluronic acid (HAc), and silk.

Silk is biocompatible and degrades slowly. However, it lacks osteoconductivity and has to be supplemented with osteoinductive features [69].

Silk scaffolds, for example supplemented with HA [69], showed enhanced biocompatibility and higher mechanical strength. In the case of addition of silk/HA core/shell nanoparticles, the best mechanical properties and biocompatibility were found for scaffolds with 40 % of silk/HA core/shell nanoparticles (NP); in the case of 60 % silk/HA core/shell NP, the mechanical properties decreased [69]. A tripolymer scaffold made out of chitosan, collagen, and hyaluronic acid was prepared by freeze-drying. The scaffolds showed good cell compatibility with MG63 cells, and the best results were found for a scaffold with a chitosan/collagen/hyaluronic acid ratio of 1:1:0.1 [70]. A scaffold made of chitosan/alginate/hydroxyapatite (1.25:1.25:1) was produced through in situ co-precipitation and was used as carrier for BMP-2 and MSC [71]. Three hydrogels made of natural biopolymers were compared towards their ability in cartilage repair. To enable cross-linking, these biopolymers were modified with methacrylate: gelatin methacrylate (Gel-Ma), hyaluronic acid-methacrylate (HAc-MA), and alginate-methacrylate (Al-MA). As control, bioinert polyethylene dimethacrylate (PEG-MA) was used. Gel-MA hydrogels were the only hydrogels of the tested hydrogels in which chondrocytes proliferated, in addition the promoted the formation of cell-secreted and mechanically functional ECM, but the cells were partly dedifferentiated [72••]. Addition of HAc-MA to Gel-MA led to significantly enhanced chondrogenesis compared to pure Gel-MA hydrogels; addition of chitosan-methacrylate also enhanced chondrogenesis but to a smaller extent [73•]. Addition of HAc-MA to Gel-MA also led to improved mechanical strength and different patients responded similarly to this component [74].

Synthetic Polymers

In the case of synthetic polymers, mostly hybrids are used, because synthetic polymers lack functionalities for biointeraction. Polycaprolactone scaffolds were produced with a ternary temperature-induced phase separation (TIPS), some additionally with porogen. The porosity of the scaffolds produced without porogen was high, but the pore size was too small for bone regeneration (20–50 μm). The use of a porogen allowed the formation of bigger pores sufficient for bone regeneration. Soaking in SBF led to apatite deposition [75].

Poly-L-lactide scaffolds were supplemented with nanosized hydroxyapatite (5 and 15 %), which improved cell compatibility towards MG63 osteoblast cells and increased expression of osteocalcin, which had a positive effect towards cell differentiation [76]. A gelatin-apatite/polylactide-co-caprolactone (GAp/PLCL) scaffold was prepared by a combination of co-precipitation and solvent casting. The hydrophilicity and osteogenic differentiation of the cells was increased by incorporation of GAp. The best results were achieved with a nanocomposite GAp/PLCL 1:6 with 70 % apatite also providing better mechanical properties than pure PLCL [77]. There are two little shortcomings in this interesting study. Firstly, the composition of the PLCL is not mentioned, which could affect the properties of the polymer. Secondly, no degradation studies were provided. Recent degradation studies of our laboratory indicate that the molecular weight of poly-caprolactone-DL-lactide (75/25) scaffold is massively reduced in the course of 2 months combined with a substantial loss in mechanical stability. This could indicate that PLCL degrades too fast in order to serve as effective scaffold in bone regeneration. Thus, degradation studies of the nanocomposites are needed.

Important Concepts

Nanotechnology

Integrating nanoparticles into the matrix system leads to altered mechanical and biological properties. Different hydrogel composites were compared concerning their mechanical properties and cell compatibilities using a lab-on-the-chip approach. The hydrogels were made of chitosan, with the cross-linker genipin and different amounts of bioglass NP. The MC3T3-E1 pre-osteoblasts preferred hydrogels with an intermediate amount of BG-NP of 12.5 wt% to chitosan with an E' value of 240 kPa and tanδ of 0.1 [78•]. Bioglass doped with 5 % zinc led to apatite formation in simulated bioglass; in the case of 10 % zinc, calcite was formed [45].

The use of nanoparticles instead of microparticles allowed to improve the biological properties. Hydroxyapatite aggregates were compared with core/shell silk/HA nanoparticles

in silk as scaffold material. The silk/HA nanoparticle system showed better biocompatibility and mechanical strength compared to the aggregates [69]. The cell compatibility of nano- and microsized bioglass was compared by incorporating them into a chitosan membrane by solvent casting. The nanosized bioglass particles led to enhanced deposition of hydroxyapatite compared to microsized bioglass particles [79]. Scaffolds made of thermoplastic urethane/HA have better tensile properties if the hydroxyapatite is introduced as nanoparticles, compared to the incorporation of microsized hydroxyapatite. Concerning hMSC differentiation, the scaffolds with the best results were made of soft thermoplastic urethane with nanosized hydroxyapatite [80•].

The dispersion of in situ-fabricated dicalcium phosphate anhydrate (DCPA) in an electrospun DCPA/PLA scaffold is more homogeneously distributed than pre-synthesized DCPA nanoparticles in DCPA/PLA scaffolds [81•].

Nanoparticles can introduce new properties into the matrix, for example, magnetic particles which interact with the magnetic field allowing to facilitate bone healing [82•]. Hybrid scaffolds of iron (Fe^{2+}, Fe^{3+})-doped hydroxyapatite (FeHA) with collagen were prepared utilizing a freeze-drying process. The FeHA was produced at different temperatures. Scaffolds with FeHA produced at 25 °C did not form hydroxyapatite; instead, amorphous calcium phosphate was formed. Still, the scaffolds had better cell compatibility than other scaffolds produced at higher temperatures. Applying a 320-mT static magnetic field (SMF) led to higher proliferation and an increase of collagen I, RunX, and ALP in these cells [83•]. Scaffolds consisting of Fe_3O_4 NP, mesoporous bioglass, and polycaprolactone (Fe_3O_4/MGB/PCL) were prepared by 3D bioprinting. The proliferation and differentiation of these systems were improved by the incorporation of the Fe_3O_4 NP [84]. Another promising system studied regarding its biocompatibility and ability to deliver clodronate consists of hydroxyapatite-based nanomaterials combined with magnetic iron oxides and multi-walled carbon nanotubes (MWCNT) prepared by wet chemical precipitation under basic conditions [85].

The use of carbon-based nanomaterials for bone regeneration is an emerging field, which was reviewed in 2013 and shows an interesting potential for future applications [86].

Supplements and Factors

Supplements and factors are interesting scaffold components allowing to alter, affect, or enhance bone or cartilage healing. Stigmastane-3-oxo-21-oic acid (SA), for example, a compound from *Lycopodium obscurum* L. (ground pine), was tested for its osteogenic potential. It could not enhance pre-osteoblast differentiation but showed a positive effect towards bone matrix mineralization [13]. Collagen matrices modified to expose galactose moieties showed better cell proliferation than pure collagen [87••].

To induce chondrogenesis of hMSCs, PLGA nanoparticles loaded with SOX9 protein and Cbfa-1-targeting siRNA were successfully applied [27••]. Collagen-microbead scaffolds with PLGA microbeads loaded with insulin can prolong survival and proliferation of chondrocytes [88]. An additional method to enhance chondrogenesis could be adding CCN2 (connective tissue growth factor) which enhances both chondrocytic differentiation and proliferation [89].

Sterilization

Sterilization is a key step for the final treatment of medical devices, implants, and scaffolds. The physical and chemical effects on the scaffold during the sterilization procedure can have an impact towards cell compatibility and scaffold structure.

Four sterilization techniques were compared towards their effects on silk fibroin: steam autoclave, dry heat, ethylene oxide, and immersion in disinfecting reagents. The sterilization methods had no significant effect on the viability of hMSCs, and all the methods were appropriate. To preserve the mechanical properties, the best method was autoclaving scaffolds in the dry state [90]. Autoclaving of silk fibroin leads to structural changes and thus to different degradation, while sterilization with 70 % ethanol does not affect the structure of silk fibroin [91]. UV radiation of silk fibroin membranes was not sufficient to sterilize them [92].

The effects of different doses of γ-radiation were examined regarding their impact towards the mechanical properties of polyvinyl alcohol/polyvinyl pyrrolidone (PVA/PVP): 50, 100, and 150 kGy. The best mechanical properties were found for hydrogels treated with γ-radiation of 100 kGy [93]. Gamma-irradiation of 50 kGy enhanced the mechanical properties of nanohydroxyapatite/polyamide66 (nHA/PA66) scaffolds, while a higher dose decreased them [94]. Sterilization did not have a great impact on the enzymatic activity of grafted alkaline phosphatase on bioglass or $TiAl_5V$ by using ethylene oxide and high-dose γ-irradiation [95].

Autoclaving of an electrospun polycaprolactone (PCL) membrane led to melting of the structure, and ethylene oxide turned the membrane into a solid film. Thirty minutes of soaking in 80 % was not enough to sterilize the membrane which was demonstrated by pre-contaminating the membrane with *Bacillus atrophaeus* as a biological indicator; EtOH was not able to kill the bacteria sufficiently. As a new sterilization method, 1000 ppm peracetic acid in 20 % EtOH was introduced and could successfully sterilize the contaminated PCL membrane without compromising the structure [96].

Conclusion

In cartilage regeneration, the dedifferentiation of chondrocytes and the differentiation of MSCs to chondrocytes are a challenging task. In order to cope with this problem, hybrid scaffolds which facilitate proliferation and differentiation of chondrocytes can be useful [73•]. In addition, the use of factors like SOX9 [88••] or CCN2 shows great potential [89]. Further details of the problem of chondrocyte differentiation were reviewed recently [97].

In the field of bioglass research, dopants allow to add new properties to the material. The use of boron as dopant enhances vascularization [42] while strontium hexaferrite allows to induce ferrimagnetic properties to treat bone cancer [41]. In addition, the mechanical properties can be enhanced by producing hybrid scaffolds, for example, with chitosan [39], or by coating the bioglass, for example, with PHBV [56].

The use of nanoparticles instead of microsized particles can further improve the biological properties [80•]. Nanoparticles are used to introduce, for example, ferromagnetic properties to allow the scaffold to be affected with a magnetic field which can facilitate bone healing [83•].

The challenges associated with the sterilization methods used for various materials differ substantially. For silk composites, the selection of the sterilization method is not critical due to its thermal stability; thus, nearly all methods are appropriate [98•]. In the case of most synthetic polymers, this is quite different; autoclaving often leads to melting, and ethylene oxide can solidify an electrospun membrane. An alternative recently introduced could be the use of peracetic acid [96].

Concerning injectable hydrogels, it would be interesting to know the internal structure of the injected hydrogel inside the body for example to examine porosity and pore size. One way to investigate these gels in vivo might be possibly by MRT, which maybe combined with the use of contrast agents like gadolinium.

Bioinspired materials have a great potential for cartilage and bone regeneration, and future research will unveil powerful approaches for this important field in regenerative medicine.

Compliance with Ethics Guidelines

Conflict of Interest Cordula S. Hege and Stefan M. Schiller declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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