CHARLES UNIVERSITY IN PRAGUE

FACULTY OF NATURAL SCIENCE

Program of study: Special chemical-biological studies
Field of study: Molecular biology and biochemistry of organisms

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Kompozitní pěnové nosiče pro tkáňové inženýrství kosti

Composite foam scaffolds for bone tissue engineering

Bachelor's thesis

Prague 2015

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Prohlášení o autorství práce

Prohlašuji, že jsem bakalářskou práci na téma Composite scaffolds for bone tissue engineering vypracovala samostatně, za použití pouze zdrojů uvedených v seznamu literatury. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze, dne 12. 05. 2015

Abstract

The goal of my work was to introduce the fast pacing field of tissue engineering with focus on bone regeneration. Tissue engineering could be a future alternative to the currently used conventional approaches that suffer from healing failures. Due to increasing demand for bone tissue replacement damaged by degenerative diseases or injuries, many laboratories have attempted to come up with solutions in a form of artificial constructs. In the present light of interest are composite scaffolds usually made of polymer and ceramic combinations. Their main advantage is that they combine elasticity and tensile strength of a polymer with bioactivity and mechanical hardness of a ceramic, while removing drawbacks of each material.

Key words: bone tissue engineering, bone, scaffold, foam

Abstrakt

Cílem mé práce bylo představit rychle se rozvíjející odvětví tkáňového inženýrství se zaměřením na regeneraci kosti. Tkáňové inženýrství představuje jednu z možných alternativ budoucí léčby kostních defektů způsobených úrazy a degenarativními onemocněními. Vzhledem k faktu, že současné způsoby léčby nejsou vždy optimální a vzhledem k rostoucí poptávce po transplantaci kostní tkáně mnoho laboratoří se snaží najít řešení pomocí speciálně připravených nosičů. Dnešním předmětem zájmu jsou kompozitní nosiče vzniklé převážně kombinacemi keramických a polymerních materiálů. Výhodou kompozitů je, že spojují elasticitu a tažnost polymeru s bioaktivitou a mechanickou tvrdostí keramiky, jejich spojením jsou eliminovány nevýhody každého z jednotlivých materiálů.

Klíčová slova: Tkáňové inženýrství kosti, kost, nosiče, pěny,

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Abbreviations

BMP = bone morphogenetic protein

SLS = selective laser sintering

BTE = bone tissue engineering

BCP = bicalcium phosphate

CAD = computer aided design

CAP = carbonated apatite

CHA = carbonated hydroxyapatite

Col = collagen

CP = calcium phosphate

ECM = extracellular matrix

FGF = fibroblast growth factor

GCSF = granulocyte colony-stimulating factor

HA = hydroxyapatite

hBMCs = human bone marrow cells

hFBCs = human fetal cone cells

HIF1α = hypoxia-inducible factor 1-alpha

IGF-1 = insulin-like growth factor 1

IL = interleukin

MCFS = monocyte chemotactic factor-1

MMPs = matrix metalloproteinases

MSCs = mesenchymal stem cells

OPG = osteoprotegerin

PCL = polycaprolactone

PLA = poly-lactic acid

PLGA = poly(lactic-co-glycolic acid)

PTH = parathyroid hormone

RANKL = receptor activator of nuclear factor kappa-B ligand

rhBMP-2 = recombinant human bone morphogenetic protein-2

RP = rapid prototyping

FDM = fused deposition modelling

SBF = simulated body fluid

SDF1 = stromal cell-derived factor 1

SFF = solid free from fabrication

SIS = small intestinal submucua

TCP = tricalcium phosphate

TNF = tumor necrosis factor

TRS = thrombocyte rich solution

TCP = tricalcium phosphate

T1C = type 1 collagen

VEGF = vascular endothelial growth factor

3D-P = three dimensional printing

1 Bone

Bone is a dynamic highly vascularized organ with complex hierarchical structure that has high remodelling capacity. Remodelling occurs constantly throughout the lifespan of an individual. Bones are made of osseous tissue, bone marrow, epithelia cells and nerves. Bone levers adequate load-bearing capacity and stores minerals. In addition to that bone is participating in homeostasis by regulating the concentration of key electrolytes in the blood and is involved in haemopoiesis (red and white blood cell formation by the bone marrow in the spongy bone) [1-3].

The osseous tissue is major structural tissue of the bone, it is relatively hard and light weight mineral matrix formed by osteoblasts secreting calcium, magnesium and phosphate ions and type 1 collagen. Bone tissue is made up of two types: Cortical (compact) bone forming exterior and Trabecular (spongy) bone filling the inside of the bone, even though they are biologically identical both have fundamentally different microstructure and function [3]. For bone tissue engineering sorting into lamellar bone and woven bone has more significance because of their role in bone regereration. Their main difference is in the organisation of collagen [4].

1.1 Lamellar bone

Lamellar bone is a mature type of a bone predominantly composed of highly organised collagen fibers into parallel or concentric arrays. One quarter of the bone is disordered material consisting of randomly oriented individual type I collagen fibers and non-collagenous proteins deposited in mineralized matrix. Collagen fibrils are formed into lamellae that are aligned next to each other or around blood vessels. This secondary bone is very resilient to distortion because in cross section the fibers are arranged in opposite directions. This is also one of the main reasons why its formation takes longer than other kinds of bone [4].

1.2 Woven bone

Woven or fibrilar bone is primary bone, usually present within an organism in small amounts and temporalilly occures in development or fracture healing. It is mechanically weaker due to chaotic distribution of collagen fibers and ground mass in between. It formes quicky, but is replaced by lamellar bone soon after in a proccess called bone substitution [4].

1.3 Extracellular matrix of a bone

Nanocomposite structure of extracellular bone matrix composes of 30 – 35% soft organic phase predominantly consisting of type 1 collagen (approximately 95%) and 60 – 70% of mineralized

inorganic material comprised of calcium phosphate. Molecules of T1C are about 300 nm long and coiling into helices that are responsible for bone toughness, viscoelasticity and fracture resistance. Triple helical structure of T1C creates spaces along the fibril that can house mineral crystals. In these grooves complexly bounded calcium phosphate is forming 1.5 - 4 nm thick and 20 – 40 nm long plate-like hydroxyapatite crystals that are responsible for bone compressive strength, rigidity and hardness. On top of it, over 200 different types of non-collagenous matrix proteins (comprising remaining 5% of organic bone matrix) like glycoproteins, proteoglycans and sialoproteins are present [2, 4, 5]

1.4 Important cell types for BTE found in bone

Osteoclasts, osteoblasts, osteocytes and lining cells are important cell types for bone regeneration.

Osteoclasts originate from hematopoietic lineage of stem cell and their direct precursors are macrophages and monocytes. [6] They are capable of both organic and inorganic bone matrix resorption [7] by synthetizing collagenase that cleaves at the Y-Gly bond in the -Pro-Y-Gly-Pro- amino sequence found vastly in T1C and capable of breaking down < 100 peptide bonds on each α-chain of the tropocollagen helix [8] and thanks to a bone enzyme called tartrate-resistant acid phosphatase (TRAP) that causing protein dephosphorization. In addition they secrete hydrogen ions and lysosomal enzymes like Catepsin K that can degrade all ECM components at low pH (maximum degradation rate at pH 4) [7]. Resorption is accompanied by release of calcium and phosphate ions from bone [3].

Osteoblasts differentiate from mesenchymal stem cells [1]. Their main task is to synthetize specific matrix proteins like collagen I, osteocalsine, osteopontine, osteonectine, bone sialoprotein, fibronectine, tetranectine, trombospondine and various proteoglycans, in other words they produce organic part of extracellular bone matrix and are responsible for its organisation as well. Additionally they secrete enzyme alkaline phosphatase, which plays role in the ossification of the organic phase. By a production of specific cytokines and growth factors (e.g. BMPs) they affect bone resorption and interestingly osteoclasts maturation and activity [3].

Lining cells are a subtype of osteoblasts, but they are more mature more differentiated: longer, flatter with a spindle like nucleus and less organelles than bone ECM producing osteoblasts. They also produce cytokines, hormones and proteins like already mentioned bone sialoprotein, osteopontine, osteonectine, alkaline phosphatase or parathyroid hormone, furthermore they express collagenase and stromelysine crucial for bone erosion. However their main function is creation of bone protective layer that plays an important role in activation of remodelling processes [3].

Estimated 20% of the osteoblasts eventually mature into osteocytes [6, 9] that are capable of physiologically significant molecular synthesis and modification of surrounding ECM. Their activity is believed to influence both bone formation and resorption through affecting other bone cells [9]. It has been proposed that osteocytes can react to mechanical strain in processes that are yet not well understood. Even though osteocytes comprise 90 – 95% of all bone cells [6], their precise role in bone remains rather clouded [9].

1.5 Bone remodelling process

Bone undergoes constant remodelling in its life time with a reckoned velocity of 5 – 10% of its volume being yearly renewed. It is a natural process of adjustment to the load pressure sites, integration and small bone tissue injury repair as well as overall regeneration. It is estimated that our bone reaches its volume and density maximum around the age of 25 years and that after 30 years slowly decreases, approximately 0,5% of bone matter per year [3]. Bone formation and resorption rates are balanced and strictly controlled processes and their disruption cause diseases like osteopetrosis (low resorption activity of osteoclasts leading to hard, dense bones), osteosclerosis (enhanced formation over resorption caused by over activation of osteoblasts) or osteoporosis (resorption predominates formation inflict low density and brittle bones) [10].

1.6 Bone healing

Bone regeneration is a multi-factorial process highly sensitive to biological and mechanical stimuli. Main biological factors represent mesenchymal stem cells, growth factors, angiogenesis and matrix metalloproteinases. Mechanical stimulations such as compression, tension, torsion and shear stress play important role in bone healing. Normal fracture healing (Fig. 1) goes through several processes: formation of hematoma, inflammation, vascularization, fibrocartilage, cartilage mineralization, woven bone formation and remodelling into lamellar bone [11].

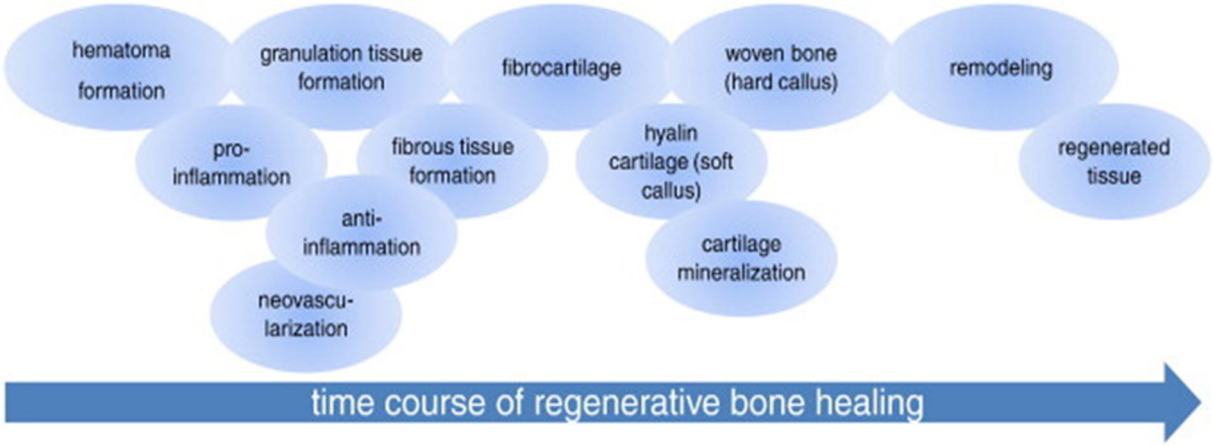
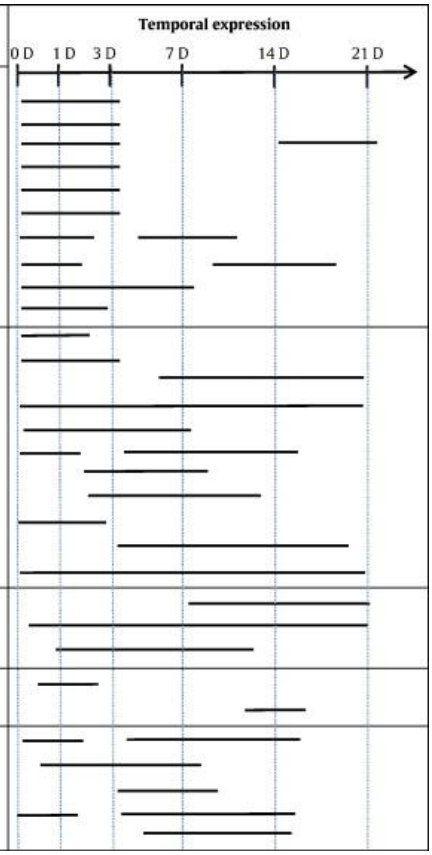


Fig. 1.Overlapping phases of the bone regeneration process [11].

If bone suffers from a trauma blood vessels and bone integrity are ruptured. Interrupted blood supply causes local hypoxia and acidosis. Macrophages and inflammatory cells immediately start inflammation mechanisms and secreting IL1, IL6 and TNFα. Importantly internal bleeding is suppressed by platelets regulating blood clot formation in the site of injury. Chemotactic factors released from platelets (i.e. GCSF and SDF1) stimulate inflammatory and progenitor cell migration and homing [12]. Oxygen deprivation triggers angiogenic HIF1α cascade and starts revascularization processes. TNFs, MCFS, OPG, RANKL induce osteoclastogenesis. Mesenchymal stem cells are progenitor cells for bone regeneration. The osteogenic differentiation of mesenchymal stem cells is regulated by interplay of growth factors and extracellular matrix properties. Among the most important growth factor stimuli are BMPs (BMP-2 and BMP-7). Other growth factors important in osteogenic differentiation include IGF-I, bFGF and TGF-beta [13]. Neovascularisation and angiogenesis is supported by VEGFs, FGFs, MMPs and other molecular stimuli [11]. For better illustration see Table 1.

Group	Growth factor	Overall action	Source for growth factor	Temporal expression
Inflammation	IL-1 IL-6 TNF α	Elicit inflammation and migration	Macrophages, Inflammatory cells, Cells of mesenchymal origin	
	SDF 1	Chemotactic factor		
	GCSF MCSF	Induces osteoclastogenesis		
	OPG	Decoy receptor of RANKL		
	RANKL	Induces osteoclastogenesis		
	COX 2			
	HIF1α	Angiogenic, hypoxic		
Growth and differentiation	TGF-β1 TGF-β2 TGF-β3	Differentiation of MSC to osteogenic cells stimulate release of other growth	Platelets, bone extracellular matrix, cartilage matrix, Osteoprogenitor cells, osteoblasts, bone extra-cellular matrix	
	BMP-2 BMP-4 BMP-7	Osteogenic factor		
	GDF 5 GDF 10		Platelets, osteoblasts, macrophages, mesenchymal cells, chondrocytes, osteoblasts	
	PDGF	Mitogenic and chemotactic factor		
	FGFs	Angiogenic		
	IGF-I	Osteogenic factors		
Angiogenesis	VEGF-b, c Ang 1 PTN	Neovascularization Angiogenesis	Osteogenic cells, Platelet	
Inhibitory	Noggin Chordin	BMP-2, 4 and 7 specific inhibitor	Osteogenic cells	
ECM	OPN Col2a1 Col10a1 CollA1 BSP		Osteogenic cells, fibroblasts	

Tab. 1 Key signalling molecules involved in bone repair [11]

Additionally cells monitor and act dependently on tissue strain and hydrostatic pressure. In early phases of bone trauma healing the strain in site of injury is high and stimulates formation of fibrocartilage callus (values higher than ≥15%). During the regeneration the strain drops to lower values due to higher mechanical stability of fibrocartilage enabling formation of bone tissue. Small tissue strain under 5% and hydrostatic pressure under (– 0.15 MPa) are optimal for direct intramembranous bone creation. High strain is usually associated with bone defects larger than critical or unstable fractures. High strain without mechanical fixation results in limited or disrupted endochondral bone [14-16].

In addition, the soft callus (fibrocartilage) maturation into bone tissue is regulated via action of multiple growth factors including BMP2 and BMP-7 [17]. Growth factors stimulate chondrocytes and other cells of MSCs lineage to proliferate and calcify the surrounding tissue. VEGF expressed in hypertrophic cartilage attracts blood vessels and stimulate formation of vascularized osseous matrix [12]. This transformation is strongly dependent on homeostatic factors RANKL, RANK and OPG, then gradually by woven bone in a presence of collagen 1, osteocalcin and alkaline phosphatase and finally woven bone remodels into lamellar bone which means restoration of the function of the original bone [11].

2 Bone defect

Trauma, neoplasms, congenital defects, infection and failed arthroplasties are causing quite large bone defects, these non-union or critical sized defects need external intervention [11]. What surgeons consider as a "critical-sized defect" is a fracture gap in tibia that involves more than 50% of the cortical diameter and is at least 1 cm in length. Critical sized defects do not have to be always critical, but their healing performances have much worse prognosis [18]. Bone is already the second most transplanted human tissue right after blood [1] and due to our aging society growing demand for massive bone grafting in the future is expected [2].

2.1 Autografts

Using autologous cancellous grafts for bone defect repair is currently the standard approach [1]. Cancellous bone was elected over cortical bone because of its osteoinductive and osteoconductive properties, because of its faster maturation, remodelation and easier revascularization [11]. However drawbacks of this technique include additional traumatic surgery (in some cases multiple) necessary to obtain autologous bone transplant from patients non-load-bearing site (e.g. iliac crest, fibula or rib), prolonged and painful recovery, higher risk of infection, potential soft tissue and bony donor site morbidity and moreover only limited amount of tissue can be harvested. Besides the quality of the transplant is largely dependent on an individual patient [1, 2, 11]

2.2 Allografts

Alternative offer allografts usually derived from human donors [11]. Success of this technique is largely dependent on the donor–acceptor interaction, their biological compatibility. Orthopedic allografts have serious limitations from disease transfer to immunological reactions. Generally the surgical bone split intervention outcome is not always as satisfactory [1]. Another issue is small number of donors and costly storage of grafts in tissue banks [11]. Food and drug administration approved and currently used allograft is demineralized bone matrix.

2.3 Xenografts

Another option used to be xenografts that are due to risk of disease transfer, immunological reactions, infection and ethical constrains, toxicity associated with sterilization and host rejection deemed unsuitable at the moment [1, 11].

Thanks to these problems there is a constant search for alternative treatment. Research towards better understanding of endogenous bone healing and mechanisms of its failure (bone regeneration can be disrupted in any stage of healing leading to a non-union) is growing. Study towards improvement of the current solutions for bone restoration is addresses by bone tissue engineers [1, 11].

3 Bone Tissue Engineering

To overcome issues connected with conventional grafting bone tissue engineering is extensively investigating scaffolds made from biomaterials that can be customized, equipped with desired properties and fabricated in great numbers [19]. Tissue engineering‘s concern is the manipulation of cells that are capable of initiating and sustaining the regeneration process using growth factors and genes, scaffolds and matrixes to switch them on to generate new functional tissue. Cells cultured on bioactive and biodegradable scaffolds or matrixes are provided with physical and chemical guidance and cues for cellular differentiation and construction of three-dimensional (3D) tissue, that may take place at a site of the injury or in an ex vivo bioreactor [19, 20] Nowadays there are hopes that it could eliminate the need for organ transplants itself by curing patients with artificial organs [1, 11, 19, 20] .

4 Scaffolds for bone tissue engineering

Scaffolds for bone regeneration are usually porous materials shaped into sheets, hydrogels or highly complex 3D structures with pores and channels (foams) [20]. These constructs are expected to promote new tissue formation via mimicking the three-dimensional environment of the extracellular matrix and provide short-term mechanical stability, adequate architecture, and surface are for cellular migration, adhesion, proliferation and differentiation into specific cell phenotypes throughout the scaffold. As the cells are filling up the construct it is desired for the scaffold to start slow degradation in order to be replaced by new tissue [1, 20]. Some scaffolds can also be intertwined with growth factors or releasing plasmid DNA containing genes for growth factors to further enhance cellular attraction and differentiation [20].

Requirements for an ideal bone scaffold [19, 21]:

I. Biocompatibility – the ability of the scaffold to support normal cellular activity including molecular signalling without any toxic effects to the tissue. For bone scaffold osteoinduction, osteoconduction and osseointegration are desirable. Osteoinductive scaffold promotes osteogenesis, progenitor cell differentiation into osteoblasts, and new bone formation. Osteoconductive means supporting bone growth (adhesion, proliferation of cells and formation of extracellular matrix on its surface and pores) and encouragement of surrounding bone ingrowth. Osseointegration is capability to integrate into surrounding bone, direct bone-scaffold contact. Additionally should be proangiogenetic to enable to form blood vessels in or around the implant within few weeks of implantation to actively support nutrient, oxygen and waste transport.

II. Mechanical properties - mechanical strength, stiffness and elasticity matching the ones of bone tissue as close as possible, but various types of bone differ greatly. Young's modulus of cortical bone is 15–20 GPa and that of cancellous bone is 0.1 –2 GPa. Compressive strength of cortical bone is 100–200 MPa and for cancellous bone 2–20 MPa.

III. Pore size – possess highly porous structure with interconnected pores over 300 μm in diameter for successful bone cell proliferation, allowing vascularisation and connection to the already existing vascular network necessary for tissue ingrowth, nutrients transport and metabolic waste exchange. Porous scaffolds equipped with both micro- and macropores perform better than only macro porous one, but increasing porosity usually weakens the mechanical properties of the scaffold.

IV. Degradability – scaffold must be bioresorbable preferably with a controllable degradation and resorption rate matching the speed of new tissue formation. Degradation speed needs to vary in accordance to its applications, for example 9+ months for spinal fusions of 3–6 months for cranio- or maxillofacial restoration.

V. Stability – maintain its properties during handling, sterilization, surgical implantation, as well as survival through physical forces in vivo or sterile environment for cell seeding

Due to large mechanical and organizational differentiations in bone, not all of the parameters can be always met. Furthermore, slight changes in porosity (pore shapes and sizes, interconnectivity or orientation), and surface chemistry, topology or architecture can significantly influence cell migration and ingrowth as well as the diffusion of nutrients and metabolic waste products. The complexity of bone regeneration makes it harder to design scaffolds with ideal composition [20, 21].

5 Design and fabrication methods

Techniques such as porogen leaching, gas foaming, phase separation, fiber meshing, supercritical fluid processing, microsphere sintering, and three-dimensional printing enable us to design a variety of three-dimensional scaffolds with different porosities and surface characteristics. Some of the methods frequently used in bone tissue engineering will be discussed below [2].

5.1 Solvent casting

Solvent casting is based on an organic solvent evaporation: either by dipping the mould into a polymeric solution and later drawing it off or by adding the polymeric solution into a mould and letting it to evaporate. Both ways result in a formation of a polymeric membrane on the mould. This method is simple, easy and inexpensive. The main disadvantage is that the solvents tend to be toxic and may contaminate the scaffold. To avoid this problem scaffold can be vacuum dried, which is time consuming or combined with particle leaching [22, 23].

5.2 Porogen leaching

Particle leaching uses liquid or solid porogens (salt, wax or sugars) to create pores or channels. Polymer solution is poured into a mould with porogen, after evaporation or cross-linking of the solvent, the porogen crystals are leached away using water. Pore size can be controlled by the amount, size or shape of the porogen. Pore diameter about 500 μm, porosity of 94-95% and desired crystallinity can be reached via this method however pore shape and interconnectivity are not very well controlled [22, 23].

5.3 Gas foaming

Exposes polymer to high pressure carbon dioxide forcing it to expands and fuse around the porogen, thus creating a continuous polymeric matrix with highly interconnected pores. This way polymer is capable of entrapping other molecules present in the mixture. Porosity of the structure depends on the amount of gas and porogen used. Technique does not require use of organic solvents nor high temperature [19, 22].

5.4 Thermally induced phase separation

Utilizes temperature change to separates polymeric solution into two phases: one with low polymer concentration (lean phase) and second with high polymer concentration (polymer rich phase). Firstly polymer is dissolved in phenol or naphthalene containing biologically active molecules, quenched and then solvent is removed from the polymer rich phase, resulting in porous scaffolds with integrated bioactive molecules. Selection suitable solvent and phase separation temperature is crucial for the formation 3D fibrous structure. Nanostructure similar to T1C can be obtained via this method. Phase separation can be combined with other fabrication methods to acquire better control over the pore morphology [22-24].

5.5 Electrospinning

Utilizes electrostatic forces of high voltage to create charged jets of polymer and to form fibres. As electric field is generated, polymer droplet placed between two electrodes overcomes its surface tension and jets towards the collector, meanwhile evaporating solvent. Micro- and nanofibers with desired orientation and scaffold with suitable structure, surface and physiological functions are generated via this method. Electrospinning offers control over pore geometry, is non-invasive and avoids use of high temperature and coagulation of substances. Use of bipolymers or cryospinning offer even higher customizability of this method [22, 24].

5.6 Fiber mesh

Fabrication of a woven or interweave fibre into 3D structure with a variable pore size by depositing a polymer solution over a nonwoven mesh of another polymer and ensuing evaporation. Scaffolds possess large surface area, however lack structural stability. Hot drying of fibres may improve scaffold’s crystallinity and structure organisation [22].

5.7 Scaffold assembly

Scaffold assembly involves spontaneous organization of a polymer thread into an ordered 3D structure. An amphiphilic peptide is deposited into an aqueous solution and as natural hydrophobic and hydrophilic interaction occurs a hydrogel is formed. This phenomenon can be controlled by pH or engineering head groups of the peptide. Compared to electrospinning self-assembly produces much thinner nanofibers with amino acid residues enabling modifications. It is performed in an aqueous salt solution or physiological media and avoids use of organic solvent, but the technique is rather complicated and laborious. Diblock or triblock ampholytes or polymer denrimers can be designed into nanofibers representing flexibility and designing potential for novel scaffolds [22, 24].

5.8 Rapid prototyping (RP)

Is a method also known as Solid free form fabrication (SFF). RP uses computer aided design (CAD) software to design a 3D scaffold that is then manufactured layer by layer by RP techniques such as fused deposition modelling (FDM), selective laser sintering (SLS), 3D printing (3D-P) or stereolithography. Reproducible scaffolds with highly controllable mechanical properties, degradation and bioactivity can be designed. In ideal scenario an image of patient's bone defect is taken and transferred into CAD model, that is then fabricated. This method offers first customable scaffolds to fit individual patient's needs, although current methods do not offer satisfactory resolution [19, 20, 22-24].

5.9 Membrane lamination

SSF-like technique that combines solvent casting and particle leaching and forms interconnected protein layers. Membranes with convenient shapes are immersed in solvent and then piled up. Each membrane is offering space for various modifications, while maintaining continuous pore structure and morphology leading to a construction of 3D polymeric foam scaffold with precise anatomical shapes. Computer assisted modelling can be used to project a template with desire morphology. This fabrication technique can be time consuming and may lead to lower pore interconnectivity [22].

5.10Freeze drying

Polymer is dissolved in a solvent in a desired concentration, solution is frozen and solvent is removed via lyophilisation under a high vacuum pressure. This creates scaffold with high porosity and inter connectivity. Pore size is controllable by freezing temperature (lower temperature producing smaller pores) and pH. Disadvantage might be longer processing and smaller pore size [19, 22].

6 Biomaterials

By a broad definition any material used in a therapeutic way to replace or repair lost function is a biomaterial. Biomaterial science is interested in materials that are biocompatible and are not passive, but actively support the efforts of the tissue to repair it-self. Biomaterials can be metals, ceramics, polymers, glasses, carbons or composite materials. They are used as moulded or machined parts, coatings, fibres, films, foams and fabrics [20]. Titanium hip joints, silicone breast implants, polyester heart valves and intraocular lenses are good examples of biomaterials known to public.

Nowadays a tremendous variety of advanced biomaterials exist, usually integrated into medical devices or implants or in contact with biological system (they are only rarely used on their own) [20]. For example bone substitutes and collagen membranes, are used regularly in regenerative dentistry as well as for bone and cartilage regeneration in orthopaedics [1]. Most often used materials in bone tissue engineering are polymers, ceramics and their composites.

6.1 POLYMERS

In biomedical engineering we can distinguish between synthetic biodegradable polymers and natural-based ones [23].

Natural polymers that have found application in bone tissue engineering for creating scaffolds are animal or plant proteins like collagen, fibrin, gelatine, silk or polysaccharides like agar, starch, alginate, chitosanes or their corresponding derivatives such as demineralised bone matrix and decellularised ECM or tissues (e.g. urinary bladder submucosa, porcine heart valves or human dermis) that are containging more than one type of macromolecules. For example small intestinal submucosa (SIS) includes in addition type I collagen, GAGs and couple of growth factors [1, 23, 24].

Collagen represents the most utilized natural polymer in tissue engineering because it is a major component of an extracellular matrix as well as it is the most abundant structural protein that is widely distributed withing the mammalian body (skin, bone, cartilage, tendons, ligaments, and blood vessels) [1]. Its stability in vivo, hydrophilicity and pore stucture make it an excellent material for cell deposition. Its ability to be converted into sponges, sheets or gels further enhance its capacity as a scaffold. Collagen is composed of three fibrilar peptide strands that coil around each other forming a tripple helix, these fibrils are responsible for the typical high-tensile strength, which is essential for the mechanical properties of the tissue through the interactions of fuctional groups in the backbone of the protein with ligands and other biologically active molecules, and are important for proper assembly of the surrounding environment thus influencing cell migration and adhesion, differentiation and morphology [1, 24].

A bit of a constrain can be its less controlable biodegradability, poorer mechanical properties and handling and as with other nature derived biomaterials there are concerns about potential pathogen transmission and immune reactions [24], but with the introduction of new techniques like electrospinning, phase separation or self-assembly it is possible to prepare synthetic materials mimicking collagen fibres [1]

Main advantage of natural polymers is that they already provide biological information e.g. paricular amino acid sequence like collagen type I that presents natural binding sites such as the Arg-Gly-Asp (RGD) and the Asp-Gly-Glu-Ala (DGEA) peptide sequences that are capable of modulating the adhesion of osteoblasts and fibroblasts [23] that guide cells and promotes chemotaxis, [2] cellular adhesion or simple maintaining of the differentiated state. Unfortunately quite a few of them have scale up difficulties and suffer from batch to batch variation. In addition weak mechanical properties, poor handeling, pontential risk of disease transmission and immunogenicity are of a great concern as well [2].

On the other hand synthetic bioresorbable polymers such as polyfumarates, linear polyesters (e.g. polylactic acid, polyglycolic acid), polyurethanes or polyvinyl alcohols [1] offer a wide range alternative [2] of materials produced under preciselly controled condition ensuring predictable and reproducible properties such as adjusted molecular weight, degradation time and hydrophobicity tensile strength and elastic modulus and more improstantly the purity of the material [23]. As a result of their combinations various co-polymers have been designed that possess different, possibly advanceable, properties.

Despite all the benefits there are some issues too, as the synthetic polymers possess some risks of toxicity or immunogenicity and may cause undesired influence on the cells, but mainly their degradation rate is usually very slow or none [23].

6.2 CERAMICS

Ceramics for bone tissue engineering are strong inorganic materials able to support surrounding tissue and furthermore possess osteoinductive properties, meaning that their surface support adhesion, growth and differentiation of osteoblastic cells, and are osteoconductive, promote bone formation [23, 24].

Amongst natural ceramics belong bone chips or powders, demineralised bone powders or substances like coralline [24]. Their counterparts are synthetically prepared bioactive glasses and calcium phosphates, including hydroxyapatite (HA), β-tricalcium phosphate (β-TCP) or calcium phosphate

derivatives [2, 24]. Particularly hydroxyapatite (HA), a mineral composed of a calcium phosphate based on tetracalcium phosphate and monocalcium phosphate [1], has been in the centre of attention for clinical use in bone tissue engineering, because it is well presented in bones and teeth and scaffolds containing hydroxyapatite are known for their enhanced osteoinduction, it has been demonstrated that mesenchymal stem cells migrate from the bone marrow or the periosteum to the implant, and differentiate into osteoblasts [1, 20].

Bioactive glasses (silica glasses containing calcium and/or phosphate) have the capability to rapidly form hydroxyapatite when immersed into simulated biological fluid. HA layer on the surface of a scaffold is bioactive and bonds with the tissue. Resorption of bioglass can be tailored to suits its purpose, slowly degrading and lasting for years or broken down within weeks, but faster degradation usually means lower mechanical strength. Great advantage of bioactive glasses is that they can be loaded with ions or bioactive molecule to further improve cell differentiation and osteogenesis. Main drawback is their brittleness, thus unsuitability for load bearing application necessary for bone reconstruction [2, 23].

To summerize it, ceramic and bioactive glasses have many great advanages and potential for use in BTE, countering this is the fact that these materials are often rather brittle [1, 2] and difficult for processing into high porosity needed for scaffolding [24]. For this reason current BTE has moved more towards the use and investigation of varisous polymer-ceramic composites [1] and highly porous but mechanically strong composite polymer-ceramic materials have been designed, promising new development in bone tissue regeneration [24].

6.3 COMPOSITE MATERIALS

Composites consist of more than one material, for example a tricalciumphosphate, hydroxyapatite or basic salts incorporated into a polymer [19], leading to different characteristics in comparison to the separate individual substances that have been used to create them. The polymer-ceramic composites imitate better the composition of a real bone, because of the combination of their unique properties: polymer elasticity and tensibility with the strength of an inorganic material [2].

Ceramic part allow to manipulate with degradation and resorption kinetics of the polymer by means that ceramic particles deposited in the polymeric matrix are responsible for intensifying biocompatibility and tissue integration in comparison to the polymeric surface which is more hydrophobic [19]. Ceramic alkalinity has also proved to neutralize the acidic autocatalysis of polymers like PLA, because basic HA or TCP products are capable of buffering the acidic aliphatic

polyester by-products and thus maintaining favourable environment of lower pH for cells and further advancing promise in use of composites [2, 19].

7 Composite foam scaffolds

I will focus my work on the progress in development of some of the frequently researched composites and introduce an example of an unusual scaffold.

7.1 Hydroxyapatite – collagen composites

Nowadays one of the frequently investigated composites for bone regeneration are hydroxyapatite–collagen scaffolds. Both collagen and hydroxyapatite are vastly represented in natural bone, therefore hold very important position in bone tissue engineering. Scaffolds from these material were found to be osteoconducive, bioactive and with good biocompatibility. By combining them we are hoping to obtain even better results from advanced scaffods.

One of the first composite scaffold did Chen et al. [25] who hybridized PLGA polymer with collagen microsponges using porogen (ice particles) leaching and freeze-drying techniques. Collagen microsponges with interconnected pores formed inside in the PLGA pores, giving the scaffold propeties of both synthetic and natural polymer. The group furher coated the microsponges with hydroxapatite, but did not continue with in vitro testing. Mechanical properties or porosity were not investigated and the proportion of components were experimental. Work displays creative use of the fabrication techniques and that creating complex scaffolds from multiple materials is possible.

Another pioneering works regarding Col-HA composite foams was published by Wahl at al. [8]. His group fabricated scaffolds from type 1 collagen and collagen-hydroxyapatite using a solid free form fabrication and critical point drying. Suspensions of 1, 3 and 5 wt% collagen were prepared, composites contained 70 wt% hydroxyapatite particles 15–70 μm in size. Only the scaffolds containing 1 wt% and 3 wt% collagen freezed at −30°C displayed pores over 300 μm in diameter, average pore size was 135 μm with the majority dropping to 50 μm or bellow. The microstructure of the scaffolds differed according to the collagen and hydroxyapatite content and the freezing temperature, increase in the collagen and hydroxyapatite proportion led to a denser construct with better stiffness, but smaller pore size. Mechanical properties of composites were still much lower than of the cortical and cancellous bone respectively. To compensate for that fact and enable fluid exchange and vascularisation, the group have introduced internal microchannels into the scaffolds. Aim of their work was to assess the processing criteria and gain control over the porosity,

microstrucure, biodegradation and mechanical properties of the scaffolds, which they have succeeded in.

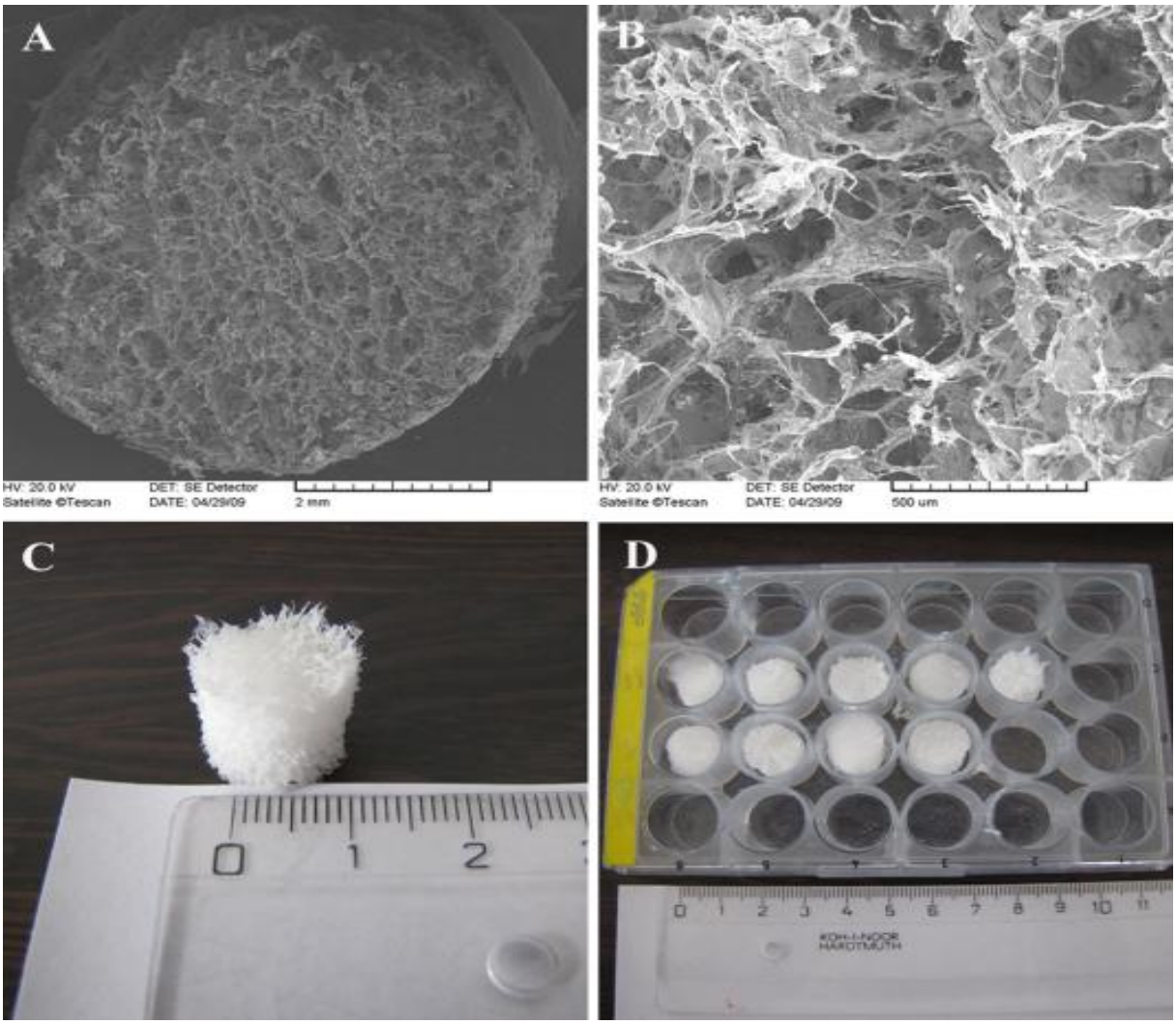


Figure 2: Coll/HA/PCL foams analyzed via SEM (A and B) and macroscopic evaluation (C and D) in Prosecka's work [26]

Sionkowska and Kozłowska [27] applied different proportion of collagen and HA than Wahl et al. [8] into the foams and compared them with lean collagen. The work assessed thermal morphological characteristics of HA in scaffolds at different temperatures. 50 and 80 wt% of nano hydroxyapatite was blended with type 1 collagen and sponges were prepared by freezing and lyophylization. The scaffolds displayed fully interconnective macroporosity and microstructure consisting of spongy collagen matrix embedded with quite large hydroxyapatite grains. Pore diameter ranging from microns to 500 µm in size. According to their observation were suitable for potential use in BTE.

The group of Shen at al. [28] has attempted to improve the fabrication of HA-Col scaffolds via applying a novel method of in situ precipitation of calcium and phosphate ionts through dual template-driven technique and freeze drying. By creating a hierarchical porous 3D foam with interconnected porosity and well-developed macropore structure with a pore size of 50–100 μm, micropores of 1–5 μm and thanks to spreading the 50–100 nm long hydroxyapatite particles homogenously within the scaffold, they have achieved improved mechanical properties of the nanocomposite. Composite exhibited more precise bonding of 2–5 nm nanohydroxyapatite particles in the internal structure than control, though it was without uniform crystallographic orientation. Control collagen did not show any hierarchical construction, but it exhibited macropores. This new method enabled to imply homogenously hydroxyapatite nanoparticles into the scaffold whilst maintaining the unique morphology and properties of the foam.

Another laboratory that was working on advancing the fabrication for bone tissue engineering was Xia et al. [29]. They empolyed a novel botom up approach to obtain collagen–apatite scaffolds. Botom up technique uses the natural princip of in situ self-assembly allowing a high degree of control over apatite content and crystal growth with freeze-casting technology. By calcium phospate coating and self-assembled collagen biomineralisation in a modified simulated body fluid (m-SBF) a dense Col-Ap composite formed. Following two freezing regimes led to a production of scaffolds with anisotropic equiaxed structure (slow and constant cooling) and aligned lamellar structure (unidirectional freezing). Col-Ap precipitates with collagen concentrations of 1.0, 2.0 and 3.0 wt% and apatite contents of 54, 35 and 18 wt% were prepared, but the apatite in the scaffolds was poorly crystallized. When apatite content increased from 18 to 54 wt%, the average pore size decreased from 106.4 to 69.1 μm and porosity from 93.2% to 91.5%. The pore size in Col-Ap-18 was larger, 40 – 160 μm on average, in comparison to Col-Ap-35 or Col-Ap-54. The Col-Ap-35 scaffold was matching the natural composition of a bone the closest and proved to have reasonably good regenerating properties after healing more than 90% of a critical sized defect in mouse.

Continuing in the same direction, Pek at al. [5] have created a porous, bioresorbable scaffold from type 1 collagen fibers extracted from rat skin and a mixture of synthetic hydroxyapatite and carbonated apatite nanocrystals by freeze drying method. The compresion test revealed the highest compressive stiffness of 37.3 ± 2.2 MPa and yield strength of 2.7 ± 0.1 MPa in T1C-CAP-HAP foam comprised of 32.5 wt% of T1C and 67.5 wt% of a nanocrystalline apatite mixture of CAP:HAP in a ratio 4:1. Grain sizes of pure CAP is 15 nm and HAP is 40 nm, but because in the trabecular bone the crystallite size is 20 nm mixture in this ratio was chosen. This composition exhibited molecular structure, crystalline phase and grain size matching that of a trabecular bone the best. They have also introduced nanopores of 50 nm and 130 nm in size for binding of adhesion proteins to trigger cell

attachement, thus making the scaffold osteoinducive, and macropores of 1–50 μm and 100–300 μm required for vascularization and osteoblast proliferation. The scaffold proved to be osteoconducive after flawlessly healing a critical-sized gap of 5 mm in the femur of Wistar rats. In compare to a control non-union fracture that did not heal at all. Additionally the scaffold healed a critical-sized bone defect of 1 cm x 2 cm in the tibia of Yorkshire–Landrace pigs, thus proving to have better osteoconductive and osteogenic properties than pain collagen and was found excellent for bone regeneration.

Because freeze drying method for scaffold fabrication as used by Pek [5] usually results in foams with relatively small pore size, which is in contradiction with the estimation of optimal pore size being greater than 300 μm for cellular infiltration, vascularization and bone in growth, and rather weak compressive stiffness and strength unsuitable for surgical handeling, Kane et al. [30] have attempt to overcome these constrains. To improve mechanical properties of Col-HA scaffolds compression moulding HA reinforcements and paraffin microspheres have been introduced into the suspension of concentrated collagen fibrils (180 mg/mL). Authors cross-linked the collagen matrix, leached the paraffin porogen and obtained scaffold with high porosity of 85–90%, interconnected pores 300–400 μm in diameter and struts of 3–100 μm in thickness containing 0–80 vol% HA whisker reinforcements. The reinforced scaffold reached an order of magnitude higher compressive modulus (up to 1 MPa) in comparison to control unreinforced collagen scaffold and even freeze-dried HA–collagen scaffolds. More over the compressive modulus was 100x greater than in absorbable collagen foams that are used clinically. Scaffolds containing up to 60 vol% HA were capable of fully recovering elastic deformatin up to loading of 50% of compressive strain for at least 100,000 repetitions, which made them suitable for surgical handeling and fixation. Scaffold have shown to be osteoconducive, capable of infiltration and differentiation of adipose-derived stromal cells and osteoinducive when implanted, promoting angiogenesis and bone formation.

Even though it was estimated that cells are very sensitive to the pore size and may require pore diameter around 300 μm, the ideal pore size nor the concentration of hydroxyapatite and collagen in the scaffold were investigated. Prosecka et al. [31] have found the optimal Col-HA composition to be made of 50 wt % HA in 0.5 wt % T1C solution, thus ratio quite different from scaffolds by some groups so far. Their study proved that pore diameter well above 300 μm is suitable for MSC differentiation, cells have prefered pore around 400 μm in size. Confirmed that pore size is negatively affected by increasing concentrations of hydroxypatite and collagen in the scaffold and that with increasing collagen content (unlike with hydroxyapatite) the scaffolds were stiffer, but still fell short on the stiffeness of the natural bone. Too low (30%) or too high (70%) percentage of

hydroxyapatite in the scaffold was preventing cell proliferation. They have also proposed, based on their data results, that a potential scaffold for BTE seeded with MCSs should be monitored for at least 28 days as 14 days have proved to be insufficient.

In further studies of Prosecka et al. [26] they have implemented polycaprolactone nanofibers into the Col-HA composite to improve mechanical properties of the construct and succeeded when Col-HA-PCL scaffold had significantly higher modulus of elasticity in compresive testing compared to the one without PCL nanofiber. In addition PCL nanofibers represents large surface area to volume ratio therefore inflict adsorption and immobilization of cells well. Next they investigated composite properties by comparing three Col-HA-PCL scaffolds: one in osteogenic media with autologous MSCs, second one in thrombocyte rich solution (leukocyte free platelet rich plasma) and final one enritched by both MSCs and TRS. 12 weeks after implantation into femoral condyle defects (0.6 cm in length and 1 cm deep) in rabbits, the best results were obtained from Col-HA-PCL scaffold enriched with both MSCs and TRS that achieved the most uniformed distribution and highest volume of newly formed bone tissue. By using human TRS in rabbit model they have also showed that these scaffolds have minimal immunological impact, making it more usable that PRP. On top of it PCL nanofibers can be used for drug delivery, thus offering potential improvement in cell proliferation. Because of a great regeneration capacity, lack of immunological response and great mechanical properties this scaffold was set as an excellent candidate for further preclinical and clinical studies.

With interesting method for scaffold design came from laboratory of Levingstone et al. [32] they have developed a layered scaffold via ‘‘integrative layering’’ freeze-drying, basically placing a layer, freezing it and than building another one on top of it, which have given them wider posibilities when designing composition, pore size or substrate stiffness in each region separately, whilts keeping the layers integrated. In this case they have succesfully replicated the healthy osteochondral tissue: first layer from T1C and HA, an intermediate layer of type I collagen, type II collagen and HA and final on containgin T1C, T2C and hyaluronic acid into a higly porous (>97%), integrated scaffold supporting MSCs infiltration and differentiation into osteochondral lineage. The average pore size in bony layer was 136 μm, in the middle 112 μm and in cartilage 126 μm. According to their study the only drawback was delamination of the weakest layer showing that the interfacial strength was greater than the tensile strength of the individual layers. And despite rather small pore sizes in the construct, they have reached such a success that their scaffold is currently being commercionalized and will undergo in vivo testing to fully assess its properties.

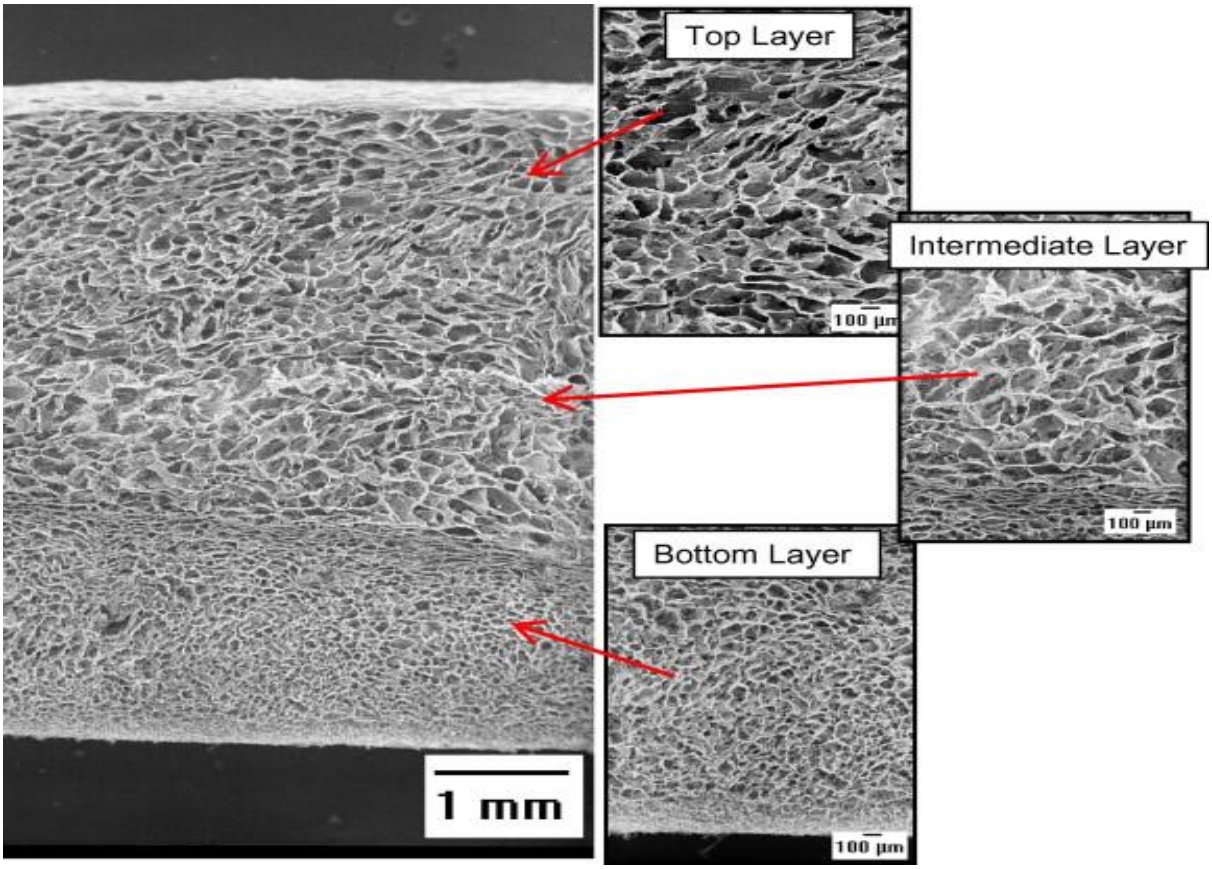


Figure 3: SEM micrographs of the three-layer scaffold the seamless integration of layers.

Because even todays composite scaffolds for BTE have unsatisfactory mechanical properties and can not withstand physiological load bearing condition in natural bone, that is why the group of Banglmaier et al. [33] have proposed to improve it by ordering collagen fibers in the scaffold by extrusion and planar fiber flow and assessed the extent of collagen fiber alignment by calculating the anisotropy index, I-ratio and angle at maximum intensity. After Banglmaier tested the tensile mechanical properties of non-extruded and extruded Col-HA, from polarized light images and Fourier transform image processing algorithm was clear that the combined extrusion and compaction increased scaffold‘s strength, elastic modulus, strain and resitance to fracture to a certain degree.

On the other hand the work of Quinlan et al. [17] went towards the drug delivery part of Tissue Engineering by developing a Col-HA scaffolds delivering rhBMP-2 in order to enhance bone repair. Using the spray drying and emulsion technique they were able to encapsulate the growth factors into the aglinate and PLGA microparticles and maintain sustained delivery of the protein for up to 28 days. In release kinetics testing both 0.5% rhBMP-2 eluting alginate and 2.2% rhBMP-2 eluting PLGA microparticle scaffold performed reasonably well, but for the purpose of bone tissue regeneration scaffold made with PLGA was found to be more suitable. After the in vitro testing the optimal rhBMP-2 releasing scaffolds, 2.2% of rhBMP-2 eluting PLGA microparticle constructs, was implanted into a

critical-sized calvarial defects in a rats. The scaffold has demonstrated an excellent healing capacity, furthermore this kind of construct can have broader application for regeneration of other tissues as well.

7.2 Composites with calcium phosphate

Because calcium and phosphate are the two main constituents of hydroxyapatite, calcium phosphates are naturally under intensive investigation in BTE. Montjovent et al. [34] were comparing the combinations of 5 wt% β-tricalcium phosphate PLA foams made via supercritical gas foaming. Cellular, acellular scaffold with demineralized bone matrix and plain TCP-PLA were prepared with 75–90% porosity, 200–500µm pore size, compressive strenght of 121.0 ± 12.1 MPa and were compared to each other and to clinically aproved βTCP Mathys and βTCP Mathys/DBM scaffolds. The most promissing results were obtained from foam made of TCP-PLA seeded with hFBCs as this scaffold performed the best in regerenation of critical size defects and drill defects in the femoral condyle in rats. Polymer degradation and cortical bone repair were followed for 12 months after implantation. The PLA-TCP scaffold seeded with human fetal bone cells represent potential especially for maxillofacial bone repair.

In similar study, Miao et al. [35] developed and evaluated HA-TCP scaffolds with 500 µm pore size and 87%, porosity prepared by polyurethane foam replica method, followed by modification with infiltration and coating of 5 µm PLGA, which worked to the benefit of the scaffold, because the bioactive HA-TCP struts were not silenced by non bioactive PLGA. Miao also proved that incorporation of PLGA increased the compressive strength of the scaffold up to 660 kPa, while maintaining the high open porosity of the foam, on the other hand after applying the freezing technique compressive strength plummeted down to 160 kPa, which was in the end sufficient to support the cell culture as migration and attachement of bone marrow stromal stem scells occured. HA-TCP scaffolds had a maximum compressive stress between 0.05-0.07 MPa, while HA-TCP-PLGA was a little bit more resistent with compressive stress maximum of 0.62–0.79 MPa. The author proposes that thicker PLGA coating would impart the higher compressive strength. On the other hand compressive modulus of the HA/TCP scaffolds was between 2.21–3 MPa, and in HA/TCP/PLGA reached even higher to 6.65 MPa. Yet nor the compressive strength or the compressive modulus of the HA/TCP/PLGA scaffolds were near the ones for human cancellous bone. The porosity of 87% and 500 µm average pore size were satisfactory and scaffold could be used for non- or low-bearing applications.

Panzavolta et al. [36] prepared gelatin/α-TCP foams via freeze drying, because of the gelatine presence the α-TCP hydrolysed into octacalcium phosphate (OCP) in proportions of 74% TCP and 26% OCP and after immension into PBS for 1 week the TCP desolved completely leaving only OCP and poorly crystalline hydroxyapatite, which is very important for the osteogenesis support. Scaffods were formed from 10 wt% gelatin solution with 9, 23, 33 and 41 wt% of TCP respectively. Scaffold showed porous interconnected microstructure, pore size 170–350 μm. This group also reported that with increasing percantage of inorganic phase the composite macro- and microporosity decreased and compression strength and Young's modulus increased. Furthermore TCP also proved to buffer the composites into more basic values of pH.

Torres et al. [37] decide to incorporate another polymer and designed β-tricalcium phosphate and hydroxyapatite construct enriched with alginate. Three types of scaffolds with different β-TCP/HA ratios and alginate coatings were developed: 80/20 wt%, 90/10 wt% and 99/1 wt% TCP/HA only or with 2% alginate. Coating with 2% sodium alginate occured under vacuum conditions. The best results were obtained from 80/20/02% TCP/HA/alginate scaffold that reached 76% porosity thanks to TCP, pores range 60 – 250 μm, and hydrophilic values, greater Young's modulus and elasticity than other coated scaffolds, furthermore these values were similar to those of natural bone. Alginate coating that was resembeling ECM of bone led to a good penetration of cells into the scaffold. It proved to be osteoconductive and osteoinductive for osteoblasts, moreover alginate does not possess any risks of disease transmition in comparison to collagen.

The aim of Arafat et al. [38] was to improve the functional performance of rapid prototyped scaffolds via biomimetic composite coating. PCL/TCP constructs were prepared by the screw extrusion system and coated with carbonated hydroxyapatite (CHA)–gelatin composite via biomimetic coprecipitation. The influence of gelatine on cell proliferation was also investigated by comparing three scaffolds PCL/TCP, PCL/TCP–CHA and PCL/TCP–CHA–gelatin, all of them with honeycomb structure, 100% pore interconnectivity, 65% porosity and pore size around 500 μm. CHA and CHA–gelatin coatings were 606 ± 106 and 821 ± 53 nm thick. CHA–gelatin coatings increased the compressive modulus of the scaffolds by 29% compared to the uncoated scaffold. What more the compression modulus of PCL/TCP scaffolds decreased by 15% under simulated physiological conditions revealed, whereas the coated scaffolds decreased by less than 10%, which was still above the compressive modulus of PCL/TCP in dry conditions. There was no difference between CHA and 600 or 800 nm of CHA–gelatin coating. PCL/TCP/CHA-gelatin scaffold had cell proliferation 2.3x times better than PCL/TCP and 1.7x time better than PCL/TCP/CHA. This scaffold possessed greater cell and tissue penetration and osteogenic defferentiation stimulus.

Another group applying polycaprolactore was Bao et al. [39], but this time with bicalcium phospates. They have managed to prepare scaffolds from PCL/BCP with adhered microspheres via gas foaming and spontaneous emulsion droplets adherence (GF-SEDA) technique and find the optimal conditions for fabrication by preparing various material combinations. Via this method BCP powder and PCL material were evenly distributed in the scaffold. The most successfull PCL/BCP foam BCP contained 25% BCL and 75% PCL, had 3D construction, pore sizes from 0.01-1000 μm, larger pores (50–100 μm) accounted for about 15.79% and large pore (100-1000 μm) for 22.26% of the total volume of pores. Compression testing revealed highly interconnected micro pores, 74% total porosity and compressive strength of 0.82 MPa, which are values within the scaffold requirements and sufficient for surgical handeling. MTT test showed that scaffold supported cell proliferation well and could be used for drug delivery or other biomedical applications. This scaffold was a great success, however further testing should follow to properly assess its characteristics.

Kucharska et al. [40] foamed 5% w/v chitosan solutions, used sodium bicarbonate as a foaming agent and than freezed dried the final mixtures. Chitosan was mixed with varying amounts of β-TCP (15%, 20%, 30%, 40% and 50% w/v), thush samples for comparing were CH, CH/15TCP, CH/20TCP, CH/40TCP and CH/50TCP. The group obtained scaffold with great pore diversity ranging from 10 to 1000 μm, Young modulus of 0.02–0.5 MPa and almost 90% inner porosity. Changing quantities of TCP visibly affecterd pore formation and shape. Degradability of the scaffold decreased and pores were more regular with rising content of β-TCP (from 10% to 50% w/v). Young modulus was greatly affected by the TCP content: 0.5 MPa for 50% TCP scaffold and only 0.02 MPa for 15% TCP—chitosan. Compressive strength was increasing along with inorganic phase percentage. The best viability of cells was observed on 15% TCP-chitosan scaffold, rest of the foams with higher TCP concent barely reached 20% of the cells on control sample. Nevertheless the cell morphology was well developed.

Through electrophoretic deposition Wen et al. [41] created a new scaffold composition based on iron foam coated with CP-chitosan mixture. Iron foam was chosen because it has similar mechanical properties as human bone, what more oxidation increased its stability. Coated with 40% nano hydroxyapatite/ethanol solution, HA displayed elongated needle-like shape and mixed with 60%nHA/chitosan-acetic acid aqueous solution the foams showed bioactivity and stability. Total porosity of 90%, pore size 500 – 800 μm and compressive modulus 1.22 MPa. Nanohydroxyapatite particles had needle like shape, 170 nm long and 8 mn wide. Author proposes more investigation to be done regarding its influence on cells.

Michailidis et al. [42] used crystalline raw cane sugar as porogen to produce open cell calcium phosphate ceramics from hydroxyapatite powder, then employed optimal compaction pressure of 250 MPa, dissolution and sintering and obtained foams with 60–75% porosity, 0.28–0.50 mm pore size and micropores of 5–7 nm after sintering shrinkage occured. HA microsturucture was bimodal with smooth and rough phase. Compressive strength and elastic modulus meassured for foams with 75% porosity reached 8 MPa and 0.37 GPA, for foam of 65% porosity even highler up to 18 MPa and 0.49 GPa. Both of the values within the estimated range for cancellous bone. Bioactivity testing, where scaffold with 75% porosity and 0.28 mm pore size performed better, revealed that both scafffolds represent excellent suitability for low load bearing BTE.

In this paper a novel injectable self-setting calcium phosphate foams (CPFs) intended for local treatments of bone defects were mixed with an antibiotic (doxycycline). Pastorino et al. [43] designed that way an innovative dosage form for bone regeneration. The material structure, drug release profile and antibiotic activity were investigated, while its clinical applicability was assessed through cohesion and injectability tests. α-TCP and 2% wt% HA were used for creating a foam with various amounts of drug, starting at 88 wt%. Doxycycline had a clear effect on both the micro and macro structure of the CPFs, owing to its role as a nucleating agent of hydroxyapatite and to a drying effect on the paste. Doxycycline-loaded CPFs presented effective release systems with interconnected macroporosity, compared with calcium phosphate cements displayed imporved kinetics of the drug, up to 55% drug was released progressively in 5 days, amount proportional to the macroporosity of the CPFs. All doxycycline-containing foams had immediate cohesion and were injectable. Moreover, antibacterial activity was observed against Staphylococcus aureusand Escherichia coli in a form that possessed less risk of creating bacterial resitence. Thus, in addition to enhancing osteoconduction and material resorption, macroporosity enables tuning of the local delivery of drugs from injectable calcium phosphates.

Nouri-Felekori's team [44] suggests that calcium phosphate whiskers are effective reinforcement of composite biomaterials. In their study composite scaffolds of varying compositions were prepared by freeze drying of 10 wt% gelatin into foams containing increasing amounts of calcium phosphate whiskers/fibrous spherulites, up to 50 wt%. Two methods were applied: treating highly crystalline beta-TCP powder with H_2O_2 solution or by precipitation (hydrolyzing calcium and phosphate ions in HNO_3 solution with added urea. First method was unsuccessful, second on the other hand resulted in scaffolds with interconnected pores ranging in 150–350 μm with homogenously dispersed 10–500 μm long plate-like whiskers and fibrous spherulites. 65–74% porosity, compressive yield strength of 4.36.2 MPa and Young modulus of 2838 MPa were achieved.

According to testing composite with 25 wt% calcium phosphate was the most suitable for following use in BTE.

7.3 Polyutherane composites

Polyurethane is a polymer that has been around for quite a while. It‘s main adventage is that it is easily convertable into a foam with customable properties, because of that it has found broand application accross many different fields. In tissue enginneering it has been used mainly as a mould for scaffold preparation, however quite recently some laboratories started to inverstigate its bioactive properties with laboratory of Zanetta et al. [45] being on of them, when they decided to characterise morphology and mechanical properties of two such foams: EC-1 with 35% open porosity and homogenous distribution of 691 µm pores and EC-2 with wider range of pore ratio, average pore size of 955 µm and 74% open porosity, both having similar densities 0.20 g cm^{-3}. In the wet condition both foams experienced a dramatic decrease in the mechanical properties. In vitro testing showed that both PU foams were colonized well by MG63 cells, more cell interaction had been observed on scaffold EC-1 compared to the EC-2, where cells adhered to the pores, but were not able to elongate and bridge them nor to form extentions to each other. Interestingly, over slower start the cell viability and inorganic phase deposition (CaP) was higher on EC-2. Both EC-1 and EC-2 successfully provided cell support, proliferation and differentiation. In vivo testing confirmed good biocompatibility as well. PU foams may become new scaffold family in BTE thanks to desired properties and easy shaping during surgical grafting.

Study of Giannitelli et al. [46] was investigating optimal polyurethane (PU) foams for oro-maxillary bone regeneration. Sponge was prepared from a polyisocyanate and a biocompatible polyester diol via one-pot reaction and possessed all the necessary propeties to be suitable scaffolds - total porosity of 65%, larger pores 306µm in size (226–408) µm and lowered later when moulding was applied), very slow degradation and furthermore Young's modulus of 24.56 ± 5.54 MPa, which got closer to the one of mandibular cancellous bone 56 ± 29 MPa. Scaffold was found suitable for hBMSC cells but further research will be conducted to fully assess the potential of the foam.

Ryszkowska et al. [47] prepared five PU with 5–20 wt% 45S5 Bioglass by combining polymer coagulation and salt-particle leaching and acquired scaffolds with porosity >70%, open pores of 100–400 µm and pore walls with micropores under 10 µm. Foams proved to be bioative in SBF and rapid coating by hydroxyapatite appeared. Composites possessed higher storage modulus than neat polyurethane. This PU-Bioglass composites were fulfilling the genereal requirement for BTE, but because this was the very first paper writen about such a combination of scaffolds, more work will have to be carried out to fully evaluate its affect on osteoblast adhesion and proliferation as well as

potential application. Additionally Ryszkowska's work proved that PU foams can be preciselly tailored to the required conditions just by small alternations in component ratios. PU polymer as well offers greater mechanical properties and improved elasticity in comparison to PGA, PLGA or PCL.

Another article considering polyurethane as potetial material for BTE was published by Dong et al. [48]. Using a foaming method a 30 wt% nanohydroxyapatite and 70 wt% polyurethane scaffold with macropores of 100–800 μm and numerous micropores, 80% porosity and 271 kPa compressive strength was designed. In vivo and in vitro testing confirmed the PU-nHA composite was not cytotoxic, provided suitable environment for cell adhesion, proliferation and differentiation. Degradation occured mainly via hydrolysis and macrophage enzymatic digestion. Scaffold was found to be convenient for potential repair of cancelour bone or articular cartilage.

Coating poly(ester urethane) foam with a calcium phosphate cement according to Peroglio et al. [49] led to a production of a filler that can be press-fitted into the defect and than hardens within it. Porous PU scaffolds with 90% porosity and 0.8–2 mm pores size were prepared by a salt-leaching. An α-tricalcium phosphate (a-TCP) cement was hand mixed with the setting liquid. Foam was than homogenously covered with a thin calcium hosphate coating was observed on the struts. Open macroporosity of the polymer foam was preserved, even with 50% volume of cement. After cement coating and setting, the properties of the scaffold were evaluated. For 5 min scaffold remained soft and viscous paste and can be safely handled and put in the cavity, but its stiffeness increases gradually over time and hardenss quickly, within 22 min. This time is sensitive for the scaffold as it can be damaged. After the cement has set the compressive strength and fracture energy increased (up to 50 MPa), almost reaching the bottom of human cancellous bone compressive modulus. On top of it, the total porosity, resorption rate and compressive strength can be tailored by the amount of cement introduced in the PU foam and adjusted to the patient's needs. These scaffolds will undego further research but are very promising for spongy bone repair.

8 Conclusion

The use of biomaterials appears to be a promising alternative to the currently applied invasive bone grafting methods. The intensive researching is bringing its results as all of the groups proved unconditionally that composite scaffolds have properties more suitable for bone regeneration than single material constructs employed before. Composites in the first works displayed reasonable biocompatibility, however possessed rather small pore size averages and weak mechanical strength, which was addressed by following studies that were assessing suitable polymer and ceramic concentrations, developing novel fabrication methods and advancing the older ones, bringing out improvement in both porosity and resistance against mechanical stress. In these works growing

knowledge and trend of more complex composites with microporosity or equipped with e.g. whiskers or microspheres is apparent. In the present studies multiple constituent composites are designed and many groups observed healing of critical sized defects in animal models. Researchers have got further in healing capabilities of composites by moving towards more complex scaffolds with not only supportive function, but serving as drug delivery systems with modified surface by growth factors. Another direction seems to be aim to obtain injectable or press-fitted scaffold that are customizable to the individual patient and some of the groups achieved excellent results. Surely there is a space for further improvement, but in my opinion, these „smart“ scaffold hold a great potential and I believe that we can expect some clinical applications soon.

What still remains a bit of a constrain, as already in early publications observed, is rivalry between stiffness and porosity. Increasing one or both of the components leads to a denser scaffold structure, but reduces the porosity and pore size. Compression testing revealed that especially ceramic content affected the material‘s stiffness significantly. Along with the increasing amount of e.g. calcium phosphate scaffolds tend to be less elastic, but with the higher compression strength. The same phenomenon was observed by many regardless the materials used for the composite. Increasing the scaffold hardness by means of higher polymer or ceramic content or gaining larger pores and better porosity at the expense of stiffness and compressive strength seems to be the main struggle of the bone tissue engineers, therefore to find a balance in polymer and ceramic ratio appears to be very crucial.

In my opinion I would have rather focused on investigation chemical properties as osteoinduction and osteoconduction, suitable porosity and degradation than mechanical ones like stiffness, because outer support like splints can be applied in the initial phases of regeneration, thus allowing constructs with low loading capabilities but excellent healing capacity of bone to be applied. It has been proved that uniform macro pores, high integrity and micro pores on walls advance osteogenesis and improve bone growth thanks to increased surface area for protein adsorption and attachment points for osteoblasts.

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