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Nanostructured bone-like scaffolds for restoration of trabecular bone remodeling capability

M. NOWAK[1]*, I. FIRKOWSKA[2], and M. GIERSIG[2]

[1] Division of Machine Design Methods, Poznan University of Technology, 3 Piotrowo St., 60-695 Poznań, Poland
[2] Department of Physics, Freie University Berlin, 14 Arnimallee, 14195 Berlin, Germany

Abstract. This paper presents the theoretical study about carbon nanotube substrates for tissue engineering and its applications. Because the replacement of bone tissue with artificial tissue can violate the remodeling process completely, the artificial material should not only consist of the same material properties, but also exhibit other characteristics which are equally important and need to be taken into consideration. These are above all the mechanosensation. Besides replacing natural tissue, the nanostructured scaffolds presented in the paper can help the tissue growth by stimulating this process. The developed trabecular bone remodeling simulation method responsible for the nanostructured scaffold behavior is implemented here. Thus, the nanostructured bone-like scaffolds reflect the remodeling capability of the biological system, not only due to their application as replacement of natural tissue, but also due to their effects in the field of mechanosensation.

Key words: bone remodeling, biomechanics, nanostructured scaffolds.

1. Introduction

Advances in computing as well as measurement instrumentation have recently allowed for the investigation of a wider spectrum of biological/physical phenomena in mechanically stimulated trabecular bone remodeling. Currently, the major focus of biomechanical research is the creation of numerical models of whole elements of biological entities. Many elements, including the biological processes occurring in living tissues, can be efficiently simulated. Trabecular bone surface adaptation has been studied intensively due to a number of reasons. Catastrophic bone failures as well as osteoporosis are leading reasons for the scientific interest in trabecular bone remodeling. Bone tissue is composed of a network of beams called trabeculae which allow bone to withstand a wide range of loads. The length of a trabecula is one or two hundred μm whereas its diameter is about 50 μm. Thus, effective trabecular bone restoration needs investigations in the range of meso- or even nano-scale. The first step in this kind of tissue engineering is to produce a scaffold, serving as a base for the tissue growth. The nanostructured matrices are an interesting alternative for the biodegradable artificial tissues. These materials not only replace the natural tissue, they can also help the tissue growth or even stimulate the growth at the same time.

2. Methods

2.1. The trabecular bone remodeling process – the structural adaptation model.

Huiskes's and Ruimerman's [1] regulatory model is the model of bone remodeling on which this work is based. The primary foundations of the model are the regulatory mechanisms within the bone structure. It is generally accepted that trabecular bone structure is a result of load adaptive bone remodeling. It is also widely assumed that mechanical stresses and strains influence the remodeling process and subsequently the structure and strength of bone. This adaptive response is referred to as Wolff's law [2]. Although the basic concepts of Wolff's law are generally accepted, the mathematical laws relating to the bone vs stress/strain relationships are still under investigation.

There are many models of bone remodeling [1, 3–5] used for the adaptation simulations of the bone with the assumption of the linear elastic material model. Justification by experimental investigations indicates that taking into account the real bone topology, the bone tissue can be treated as a linear elastic material [1, 3]. Such an approach can be considered very useful, especially when details of mechanical stimulation are discussed.

Figure 1 presents the 'regulatory model' conception based on clinical observation of trabecular bone tissue behavior. The main assumption of this model is the existence of homeostasis (perfect balance between bone gain and loss). This equilibrium can occur only in the presence of mechanical stimulation. The network of osteocytes plays the role of sensors of the mechanical energy distribution along the trabecular bone tissue. Moreover, mechanically induced osteocyte signals explain osteoclast and osteoblast activity in Basic Multi-cellular Units [6]. Thus, the regulatory mechanism is responsible for the remodeling process in the trabecular bone on the level of a single cell. The model used here postulates strain energy density (SED) as a scalar measure of mechanical stimulation and the distinguished value of SED, corresponding to bone remodeling homeostasis. The model assumes also that small deviations from this distinguished energy value do not substantially influence the remodeling phenomenon (lazy zone). Only significant changes in the mechanical stimulation result

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*e-mail: Michal.Nowak@put.poznan.pl

in bone loss or gain (left and right part of the plot respectively). This process is reflected in the modification of osteoclast and osteoblast activity.

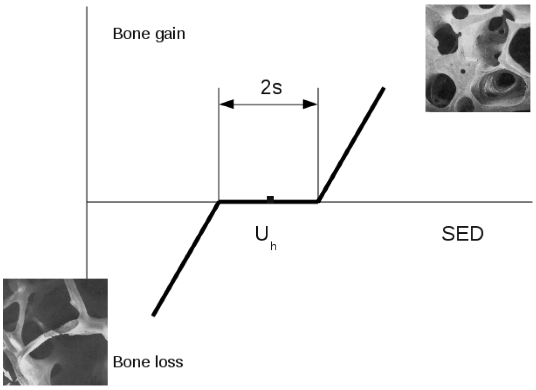

Fig. 1. Huiskes and Ruimerman remodeling regulatory model scheme

The ‘regulatory model’, presented in Fig. 1 is described according to [6] by the following equations:

$$\frac{dE}{dt} = C_e\left[U - (U_h + s)\right] \quad \text{for} \quad U > (U_h + s)\,, \tag{1}$$

$$\frac{dE}{dt} = 0 \quad \text{for} \quad (U_h - s) \leq U \leq (U_h + s)\,, \tag{2}$$

$$\frac{dE}{dt} = C_e\left[U - (U_h - s)\right] \quad \text{for} \quad U < (U_h - s)\,, \tag{3}$$

where E denotes Young modulus of the tissue, U_h is the SED value corresponding to homeostasis of bone loss and gain, $2s$ is the size of the lazy zone and C_e is a constant value.

The phenomenon of trabecular bone adaptation thus has two important attributes. Firstly, mechanical stimulation is needed to conserve the rebuilding balance, even on the level of a single cell. Secondly, the process of resorption and formation occurs only on the trabecular bone surface. These factors together with the ‘regulatory model’ conception were the base of the generic, three-dimensional system for bone remodeling simulation [7]. The developed system mimics real bone geometry evolution where the volumetric finite elements mesh and the surface of the trabecular network is controlled during the remodeling simulation.

The crucial feature of the trabecular bone remodeling phenomenon is the continuous change of the tissue structure, where there is thus no pattern for the bone structure.

As an illustration of this statement, the Miab [5] project data was used. The bone of a female Wistar rat was scanned with the use of the micro CT method with a resolution of 21.8 microns. The FEM model based on these data sets was prepared. To produce the model, 50 slices were used and the resulting mesh contains 2 million tetrahedral elements. On the left hand side in Fig. 2 one can observe a section of 1 millimeter of the whole bone. The data sets presented here comes from the control group, so the change of the bone architecture can be treated as an illustration of the normal structure evolution. The first one contains the images of the healthy bone of a 10 month old rat, whereas the second one contains the images of the same bone, 14 weeks later. The form of the structure is different, but the function remains the same. Thus, if there is the need to replace a bone structure, the artificial material should not only have the same material properties, but also consider other important issues, most importantly the mechanosensation. Replacement of the bone tissue with the artificial one violates the remodeling process completely. The artificial structure is unable to change the trabecular bone topology, and is thus not able to react to the “change of the function” described in Wolff’s Law.

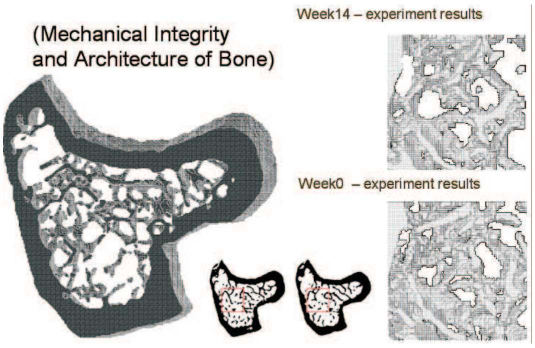

Fig. 2. Trabecular bone remodeling – Miab project data – experiment and simulation of trabecular bone adaptation after Ref. 7

2.2. Carbon nanotube substrates for tissue engineering applications. The method used here is called the layer-by-layer assembly process [8, 9]. Briefly, latex micro particles with a diameter of 1.71 μm were first slowly applied on the water surface and subsequently self-assembled into a hexagonally closed pack monolayer. After deposition of such a formed monolayer on a silicon substrate, the adsorption of positively charged polyelectrolyte and negatively charged carbon nanotubes took place.

The resulting nanotube-based matrix is depicted in Fig. 3. After the chemical delamination of the latex particles, a very regular structure is obtained, which is illustrated in Fig. 4. The free standing nano matrices obtained by this method were used for the experimental testing of the biocompatibility. The biocompatibility of the nanotube substrates were studied very widely. This problem was also the subject of the research in the team of Prof. Giersig [9].

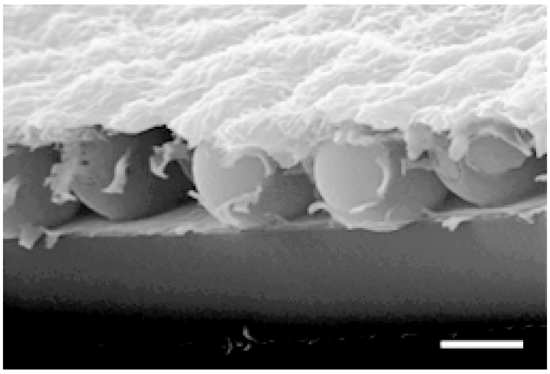

Fig. 3. Scanning electron microscopy micrograph of a multiwalled carbon nanotubes matrix before chemical delamination of the latex (Scale bar: 1 μm after Ref. 9)

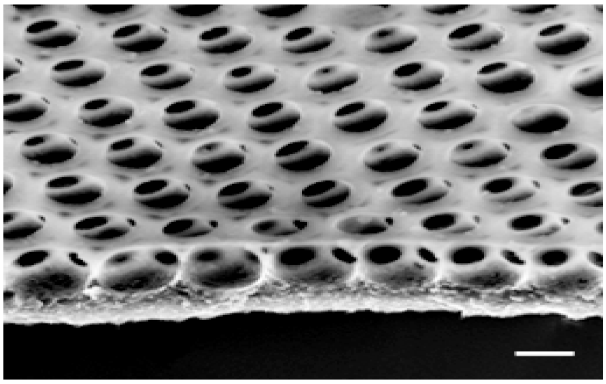

Fig. 4. Scanning electron microscopy micrograph of a multiwalled carbon nanotubes matrix after chemical delamination of the latex (Scale bar: 1 μm after Ref. 9)

The obtained results indicate excellent cells responding to the micro- and nanotopographical cues present on the nanostructured surface. In addition, the nanotube structures show no toxicity for the other tissues. The osteoblast cells growing process on the free standing nanomatrix described above is depicted in Fig. 5.

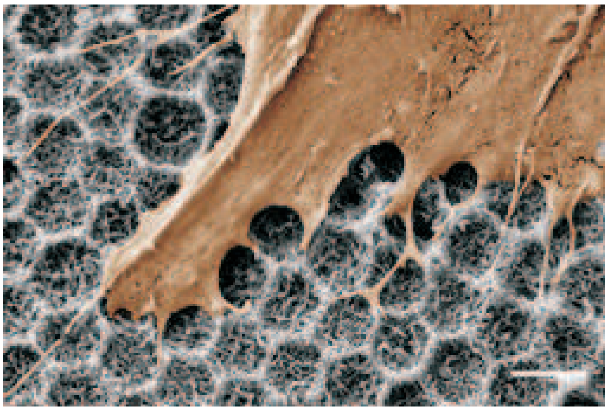

Fig. 5. Scanning electron microscopy pictures showing the morphology of osteoblast-like cells (Scale bar: 1 μm after Ref. 9)

The structural optimization of the nanostructured scaffold as well as geometry description in accordance with biological principles will provide a synergetic effect. Firstly, the biocompatibility will enable the tissue growth on a cellular level. Secondly, the mechanical properties of the nanostructured scaffold will enable the occurrence of the trabecular bone remodeling process.

3. Results

3.1. Nanostructured scaffolds strength properties improvement demands. We can now summarize the previous discussion and describe the suggested properties of the nanostructured bone-like scaffolds:

- The nanostructured bone-like scaffolds should behave like a real trabecular bone tissue – the mechanical properties of the nanostructured bone-like scaffolds should be similar to the properties of the trabecular bone tissue.
- Contrary to other approaches, the nano particles should act as bricks in the remodeling process – mechanosensation is crucial for the remodeling process and must be also present in the modified bone structure – not to replace the bone tissue, but to help in the remodeling process.
- Due to proven biocompatibility of free standing nano matrices structures it is possible to support the remodeling process – the nano particles should be of proper size and shape to enable of structural consolidation and separation during the structural evolution.

3.2. Numerical analysis. To fulfill the above presented demands, a numerical simulation was performed. The virtual geometrical models of the multiwalled carbon nanotubes matrix after chemical delamination of the latex were based on the data presented by I. Firkowska. The geometrical model of the nanotubes matrix is depicted in Fig. 6. To simplify the analysis the continuous material model approach was chosen [10]. For the numerical simulation, the linear elastic material model was used, where the Young's modulus $E = 4$ GPa and Poisson's ratio $\nu = 0.3$ were assumed. Such a simplification is allowed because of size of the modeled structures and is in agreement with the experiment [9]. For the mechanical tests, two load cases were chosen – shear and compression of the structure, as a representation of possible loads in the multiwalled carbon nanotubes matrices. The same load cases were tested for the trabecular bone structure with the assumed continuous material model with the parameters of respectively $E = 2$ GPa and $\nu = 0.3$. For the computations, the Finite Element Method (FEM) system ABAQUS was used. The FEM meshes both for the continuous bone structure model and for the exact geometrical model of the nanotubes were prepared with the use of tetrahedral finite elements. The results of the simulation tests are summarized in Table 1. The reference displacements of the multiwalled carbon nanotubes for both load cases were the trabecular bone model (100%). The computed displacements of the multiwalled carbon nanotubes model are much larger than the displacement of the similar structure of trabecular bone. Because the mechanical properties of the nanostructured bone-like scaffolds should be similar to the properties of the trabecular bone tissue, the initial structure was optimized using the optimization tool Cosmoprojector [11].

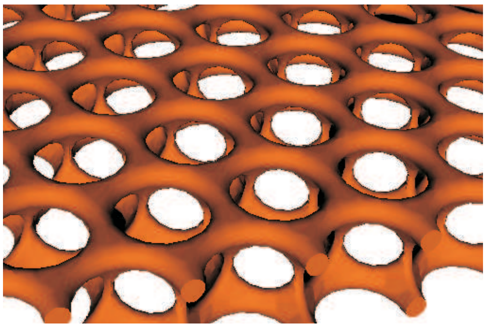

Fig. 6. Geometrical model of the multiwalled carbon nanotubes matrix after chemical delamination of the latex (translated to VRML format)

Table 1
The results of the multiwalled carbon nanotubes matrix shear and compression virtual mechanical tests (the initial model)

Load case	Shear	Compression
Trabecular Bone – continous material model	100%	100%
Nanotubes – exact geometrical model	1701%	566%

Table 2
The results of the multiwalled carbon nanotubes matrix shear and compression virtual mechanical tests (the modified model)

Load case	Shear	Compression
Trabecular Bone – continous material model	100%	100%
Nanotubes – exact geometrical model	66%	277%

Such an approach allows mimicking the real biological process of bone formation towards the artificial structures in detail, because the structural optimization process is based directly on the remodeling phenomenon. In the simulations, the strain energy density (SED) distribution resulting from the FEM computation was used as a remodeling criterion. It was assumed that local adaptation was a function of SED with a "lazy zone", as described in [6]. If the SED value in the structure was higher than the assumed level, surface adaptation occurred adding the tissue material on the surface. If the SED value in the structure was lower than another assumed level, surface adaptation occurred again, but this time, removing the material. If the SED value was between the two levels described above, no adaptation occurred and this represents the lazy zone.

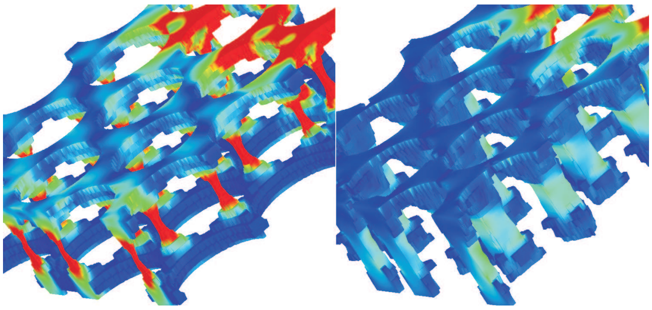

Fig. 7. The modification of the initial multiwalled carbon nanotube structure using the principle of constant strain energy density (left – original structure, right – modified structure)

The structure resulting from the computations has mechanical properties similar to the real tissue and should behave in the same way, when loaded (Table 2). The method allows treating the artificial material (the multiwalled carbon nanotubes) as an evolving structure, when the loaded structure reaction becomes similar to the real tissue. The modification of the initial multiwalled carbon nanotubes structure is presented in Fig. 7.

3.3. The carbon nanotubes matrix modification. Now the question of how to realize the proposed modification of the multiwalled carbon nanotube matrix in practice arises. The solution could be reactive ion etching (RIE) [12]. The RIE concept utilizes dissociation and ionization of neutral gas in an alternating electrical field. Accelerated ions collide with molecules fracturing them into radicals. Mechanical sample's bombardment with constantly accelerated oscillating ions in a changing electrical field is primarily causes a physical etching and heat transfer of the sample. At the same time, highly reactive chemical radicals diffuse to all surfaces in a chamber and react with them (chemical etching). The RIE process is almost linear. The initial state of the unetched and etched mask of latex nanospheres is presented in Fig. 8.

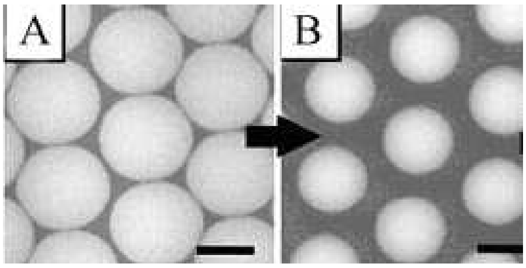

Fig. 8. The process of reactive ion etching. A – The initial state of the unetched mask of latex nanospheres. B – etched mask of latex nanospheres after Ref. 12

The results of the process of reactive ion etching is in line with the modification of the initial multiwalled carbon nanotube structure obtained by use of the constant strain energy density principle, presented in Fig. 7.

4. Conclusions

The developed trabecular bone remodeling simulation method to simulate the nanostructured scaffold behavior was implemented. The nanostructured scaffolds finite elements modeled with special emphasis to finite element mesh correctness were prepared. Analysis of the possible role of the nanostructured scaffolds in the trabecular bone remodeling process was performed. The modification of the multiwalled carbon nanotubes structure was proposed including the necessary technology.

The results of the work can be summarized as follows:

- The nanostructured bone-like scaffolds can behave like a real trabecular bone tissue.
- The nano particles can act as bricks in the remodeling process.
- Due to proved biocompatibility of MWNT-based structures it is possible to support remodeling process.

The nanostructured bone-like scaffold is able to restore the remodeling capability of the biological system including mechanosensation. Clusters of the multiwalled carbon nanotubes can be incorporated in the cancellous area and treated as bricks in the remodeling process. Due to the biocompatibility of free standing nano matrix structures, it is possible to support the remodeling process including mechanosensation (which is crucial for the bone remodeling process).

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