Design and rapid prototyping of DLC coated fractal surfaces for tissue engineering applications

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Abstract. Several medical devices (both implantable and for in vitro diagnosis) benefit greatly from having microtextured surfaces that help to improve and promote phenomena such as osteointegracion and cell / tissue growth on the surface of a device. Normally, the use of abrasives or chemical attacks are employed for obtaining such surface microtextures, however, it is sometimes difficult to precisely control the final surface characteristics (porosity, roughness, among others) and consequently the related biological aspects.

In this work, we propose an alternative process based on the use of fractal surface models for designing special surfaces, which helps controlling the desired contact properties (from the design stage) in multiple applications within biomedical engineering, especially regarding tissue engineering tasks. Manufacturing can be directly accomplished by means of rapid prototyping technologies. This method supposes a focus change from a conventional “top-down” to a more versatile “bottom-up” approach.

Finally, in order to improve the possible biological response, the surfaces of the designed devices were coated with hydrogen-free amorphous carbon (a-C) thin films, known to be highly biocompatible materials. The films were deposited at room temperature using the vacuum filter cathodic arc technique. Our first prototypes have helped verify the viability of the approach and to validate the design, manufacturing and coating processes.

Key words. Fractal, scaffold, tissue engineering, rapid prototyping, diamond-like carbon.

1. Introduction: Fractals in biomedical engineering
Fractals are rough or fragmented geometric shapes that can be split into parts, each of which is (at least approximately) a reduced-size copy of the whole. The term fractal was coined by Benoît Mandelbrot in 1975 [1] and derives from the Latin fractus meaning “broken” or “fractured”. The term “fractal” is used to describe complex geometries that are too complex to be described in conventional Euclidean terms, with properties like self-similarity and defined usually with simple recursive procedures.

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Since the early works linked to fractal geometry in 1975, it became clear that they could be used for describing the geometries, patterns and roughness of natural objects [1-2]. Although fractals are commonly considered to be infinitely complex (due to their usual recursive definitions) “approximate fractals” are easily found in nature, which usually display self-similar structure over an extended, but finite, scale. By limiting the steps applied in a recursive definition of a conventional fractal, approximate fractals can be obtained, which mimic complex natural geometries. Natural objects that are approximated by fractals include clouds, mountains, lightning bolts, coastlines, snow flakes, various vegetables and several corporal geometries.

During the last decade, increasing attention has been paid to using fractals for promoting modeling, design and simulation tasks in several areas of Bioengineering. The most remarkable are discussed below:

- **Modelling the behaviour of microorganisms.**
  Several studies have been reported on the use of fractal models for describing the growth and expansion rate of bacteria and for evaluating the dynamics of coexisting species of microorganisms [3].

- **Modelling complex organisms and their systems.**
  Regarding complex organisms (including human anatomy) fractals have been applied to modelling systems of pulmonary and blood vessels and vascular networks, as well as for carrying out subsequent fluid mechanics simulations [4].

- **Modelling the surfaces of organs and tissues.**
  Recent interest has appeared in the use of fractals for mimicking the surfaces of organs and tissues and thus improving the designs and *in vivo* performance of several prosthetic devices [5].

In this study, we focus on the design, manufacture and biological conditioning through a surface coating of fractal surfaces for obtaining special rapid-manufactured scaffolds with controlled features such as roughness, waviness, skewness or fractal dimension, among others.

**2. Benefits of fractal scaffolds and current limitations**

Several studies have focused on the importance of surface microtexture, roughness and pore size for promoting positive effects in all kinds of biodevices, from implantable prosthesis to scaffolds for cell and tissue growth. These textures have a significant influence in osseointegration of prosthesis, cell proliferation and tissue growth given that those cells and tissues seem to be more “comfortable” when faced with biodevices with similar surface properties [5-6].

However, the process of introducing desired roughness on the surfaces of biodevices is still mainly linked to carrying out machining operations, laser processing or chemical attacks. In all these cases, post-processing operations could be difficult to control. The use of fractal models for mimicking such natural surfaces can prove to be useful for design tasks. The process explained in the following section allows defining and controlling the texture and roughness of surfaces from the design stage, with help of computer-aided design tools. Its application to obtaining fractal scaffolds is shown as example.

The mentioned computer-aided design, calculation and manufacturing technologies (CAD-CAE-CAM), have become essential tools for developing products. They allow 3D geometries and alternative designs (to which calculations of stress, deformations, ergonomics, dynamic response can be applied) to be rapidly manufactured. Moreover, these technologies can be employed to test materials and designs before manufacturing. The multiple benefits of these technologies can be employed for developing conventional products could prove to be extremely beneficial when applied to designing biomimetic and personalized scaffolds for tissue engineering, although some limitations exist.

Some enterprises already provide their customers with scaffolds, designed with the help of CAD tools, however they are limited to conventional design operations from such software (holes, grooves, extrusions) and the related scaffolds do not provide the desired complexity.
Additionally the use of CAD tools can be especially beneficial when combined with a new set of manufacturing techniques and technologies that have appeared in the last two decades called “Rapid prototyping and manufacturing technologies”. These technologies help to address market requirements in an ever more customised way and to provide support for research work where physical models (or prototypes) are needed for tests and trials that are optimised in terms of time and cost.

They are usually based on manufacturing processes that add layers or “layer manufacturing technologies” (like “laser stereolithography”, “3D printers” or “selective laser sintering”), in rapid shape-copying processes, or in manufacturing processes through the elimination of material (such as in high speed numerical control machining). The different technologies available allow prototypes to be obtained rapidly in a wide range of metallic, ceramic or polymeric materials with remarkable precision [7-8].

These technologies enable physical parts to be manufactured in a short time (a couple of hours or a few days) directly from the designs made with the help of computer-aided design, calculation and manufacturing programs or “CAD-CAE-CAM technologies”. They greatly help to optimise design iterations, contribute to early error detection and speed up production start-up.

3. Designs of fractal scaffolds

We propose and explain in this section the use of mathematical fractal models for designing the surfaces of the scaffolds and the use of these surfaces, together with CAD programs, for obtaining three-dimensional virtual prototypes of the scaffolds for subsequent automatic manufacture. In this way, parameters such as roughness, waviness, skewness can be controlled from the design stage and adapted in a more efficient way to the requirements of the damaged organ or tissue.

For our initial designs we have selected a fractional Brownian fractal surface model, which has previously proved to be useful when carrying out designs of natural surfaces [2]. The following equation gives the height “z” of the surfaces, when assessing the function over a grid of points given by their (x,y) coordinates. The model uses several random functions (Ak, Bk, Ck), several control constants (λ, α, m) and an initial height function “z0” can also be introduced.

\[
z(x, y) = z_0(x, y) + m \sum_{k=1}^{\infty} C_k \cdot \lambda^{-\alpha k} \cdot \sin(\lambda^k [x \cdot \cos(B_k) + y \cdot \sin(B_k) + A_k])
\]

Figure 1 shows the result of evaluating a fractional Brownian fractal function over a grid of 60 x 60 points (corresponding to a scaffold of 30 mm x 30 mm) and the influence of introducing changes in the control parameter “α”. Figure 1 (a) corresponds to α = 0.8 (fractal dimension around 2.2 with maximum roughness depth reaching 1.2 mm) and Figure 1 (b) corresponds to α = 0.2 (fractal dimension around 2.8 with maximum roughness depth reaching 2.5 mm). In these examples we have used \(z_0(x,y) = 0\), what leads to positive and negative values of z in the images of Figure 1, as the z-axis shows. The calculations have been carried out with help of Matlab software (Mathworks, version R2009) and the data obtained are stored in three-column matrices [X, Y, Z]. The command “surf” helps to represent the surfaces linked to the mentioned matrices.

Once the Matlab surfaces have been obtained, the information stored in the form [X, Y, Z] can be converted into .stl universal format, so that the surface can be recognized and imported with a CAD program, for additional design operations (i.e. providing the surface with a thickness different than zero, copying the surface atop from a previously designed geometry). The explained process is currently patent pending, both for two-dimensional fractal surfaces with application in tissue engineering [9] and for three-dimensional fractal structures with application in biofabrication [10].

The rapid manufacture of such fractal scaffolds and the use of DLC coating for promoting their biocompatibility are described in the following section.
Figure 1. Designs of fractal surfaces with different roughness and fractal dimension. (a) $\alpha = 0.8$, fractal dimension around 2.2 and (b) $\alpha = 0.2$, fractal dimension around 2.8.
4. Manufacture of fractal scaffolds
In spite of the fast incorporation of rapid prototyping into product development methodologies, these new types of technologies are still in their initial stages of development, hence their applications in bioengineering (and especially in tissue engineering) are continuously evolving [11-12].

In this study we propose the use of a rapid prototyping technology (laser stereolithography) for directly manufacturing fractal scaffolds from the previously explained designs. Some enterprises already provide their customers with rapid-prototyped scaffolds, however they are normally designed using conventional CAD design operations (holes, grooves, extrusions) and the related prototypes do not provide the desired complexity. The design and manufacturing approach proposed here provides additional control tools and helps to obtain biomimetic scaffold designs, which can be directly manufactured. Figure 2 shows an example of a physical prototype of fractal scaffold manufactured through laser stereolithography in epoxy resin (Accura 60, 3D Systems), directly from the CAD file of the part. It has been manufactured using the SLA-3500 machine from (3D Systems) which can manufacture details down to around 150 µm.

It is important to note that using a layer manufacturing technology allows to obtain very complex geometries, even with inner details and porosity, in many cases unable to be manufactured using conventional top-down approaches such as machining or chemical attack. Aspects like roughness, waviness, skewness or fractal dimension can be precisely controlled from the design stage and adapted in a personalized way to the requirements of the application.

Although the photopolimerizable epoxy resin used for the first trials in not a medical grade polymer, and therefore cannot be used for the manufacture of implantable devices, it is important to mention some recent advances in the development of biocompatible photopolimerizable resins for stereolithography, which will help in the following years to overcome this limitation [13].

In the meantime we propose two possibilities for tackling the problem of biocompatibility. Using the epoxy fractal scaffold we can obtain silicone moulds by rapid form copying for subsequent casting of medical grade polymers conventionally used for scaffolds (polyvinyl alcohol, polycaprolactone, polydimethylsiloxane). For \textit{in vitro} diagnosis and cell culture tasks, a more direct procedure through the deposition of a highly-biocompatible coating can be a more appropriate solution.

5. Diamond-like carbon coatings for property enhancement
After obtaining the prototypes, hydrogen-free amorphous carbon thin films (a type of diamond-like carbon or DLC with sp3 content below 30%) were deposited at room temperature using the vacuum filter cathodic arc technique [14].
Remarkable adhesion and film conformity were obtained in the initial trials; however some small fissures appeared in some of the sharper tips of the prototype due to the high aspect-ratio of some of the features, as shown in Figure 3, and the induced stress produced by the deposition of the a-C film. However, tips fissure problems can be avoided by the use of scalonated and smoother designs, as shown in Figure 4. DLC coatings with thickness of around 100 nm were successfully applied using the cathodic arc technique.

In any case, these initial trials helped to show the viability of successfully coating rapid-prototyped epoxy parts with diamond-like carbon coatings, which are highly biocompatible as reported in previous work [15]. Moreover the possibility of combining the benefits of amorphous carbon with some quantities of metal (especially Ag or Cu) as doping agents can lead to surfaces with enhanced anti-bacterial properties, especially useful when taking into account the possibility of implanting such devices [16-17].

It is important to note that the coating process has to be carried out at room temperature, which allows coating polymers, with glass transition temperatures around 40 – 60 °C, without inducing remarkable deformations or morphological changes. Thus, final devices, even after coating, possess the geometrical properties defined from the initial fractal model used and afterwards converted into the CAD file.

**Figure 3.** Pictures of fractal scaffold prototype: (a) uncoated, (b) after DLC coating.

**Figure 4.** Detail of a second prototype of rapid manufactured scaffold with DLC coating. Layer by layer process can be perceived in detail inside the image.
6. Conclusions and future work
We have presented a novel method for designing, manufacturing and enhancing biocompatibility of scaffolds for tissue engineering based on the use of fractal surfaces for promoting biomimetic approaches. Several designs and prototypes have been obtained for validating the different stages of the process.

Linking fractal models with CAD programs and rapid manufacturing technologies proves to be a powerful tool for precisely controlling (from the design stage) the final properties of the scaffolds like roughness, waviness, skewness or fractal dimension. Main future actions are aimed at *in vitro* validation of the prototypes obtained and at the verification of the coating influence on biocompatibility. Furthermore, it is important to explore the use of microstereolithography for increasing the accuracy of our prototypes and for enabling the application of the proposed methodology to solve additional bioengineering needs.

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