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Abstract— The design and fabrication of highly porous polymeric or composite scaffolds for tissue engineering and organ regeneration are keystones to advance in these interdisciplinary research areas. The development of biocompatible polymeric matrices with particular morphologies that promote a specific biological response for each type of cell, is strongly needed. To attain this goal, the characterization and quantification of the morphological properties of the scaffolds are necessary to correlate them to mechanical and biological properties.

In this work, micro/nanofibrous scaffolds obtained by electrospinning were characterized by scanning electron microscopy (SEM). The use of the granulometric size function, computed from the scaffold images, to develop algorithms that help the specialists in the characterization of the shape, size, stocking density and orientation of the arrays, is proposed. The obtained results show that the analysis of SEM images allows for a good characterization of fibrous scaffolds becoming a useful tool for specialists in this research area.

*Keywords* — Digital Image Processing, Mathematical Morphology, Tissue Engineering, Biomedical Polymers.

## I. INTRODUCTION

Tissue engineering is currently one of the most attractive areas of multi-and interdisciplinary research. Among other essential components, tissue engineering requires appropriate artificial extracellular matrices (ECM) in the form of highly porous scaffolds, able to regulate and stimulate the cellular functions of adhesion, migration, growth, differentiation and tissue organization (Ikada, 2006; Abraham et al., 2007). The factors governing the properties of the scaffolds are complex and include chemical and biological composition, spatial architecture, mechanical and surface properties, and degradation kinetics. Polymeric scaffolds must provide adequate mechanical properties to match that of the host tissue and to aid the differentiation of certain cells. Architectural features have also an important role to mimic the functions of the native ECM. Thus, scaffolds must allow cell attachment and subsequent migration within the matrix, mass transfer of metabolites and enough space for the development of a system of vascularization and remodeling of the scaffolds of organized tissue.

Processing of polymeric nanofibers through electrospinning has gained much attention in the last decade due to its versatility for producing a wide variety of polymeric fibers as well as due to its ability to produce fibers in the submicron range that is otherwise difficult to achieve by using conventional fiber-spinning technologies (Bhardwaj and Kundu, 2010). Depending of a number of the intrinsic properties of the solution and processing parameters, uniform bead-free micro/nanofibers are collected in a grounded target, leading to the formation of a non-woven mat.

Therefore, characterization and quantification of the internal porous microstructure of polymeric scaffolds, surface area-to-volume ratio, spatial distribution, degree of interconnectivity, and orientation of the nanofibers are of great importance to the interpretation of the biological response of in vitro and in vivo tissue growth.

Conventionally, shape analysis is performed on binary images of the scaffold. Several indicators are used to measure the object area, perimeter, radius (of a circle with similar area), roundness factor, entropy, curvature and skeleton. However, the variability of the characteristics of gray level biomedical images often prevents obtaining binary images representing the objects of interest (Glasbey and Horgan, 1994; González and Woods, 1996; Castleman, 1979). Unlike standard techniques, morphological techniques are based on concepts of geometry, algebra, topology and set theory, to characterize structural properties of images (Facon, 1996; Serra, 1982, 1988 and 1992; Dougherty and Astola, 1994; Marshall and Sicuranza, 2006). The central idea of these techniques is to examine the geometric structures in an image by overlapping it with small size patterns, whose shape depends on the form of the components to be analyzed in the images, and the information that is to be obtained from the images. In this context, Mathematical Morphology provides a satisfactory solution for the analysis of shapes. Of all the techniques of analysis that belong to the field of mathematical morphology, the most appropriate tool, to characterize the shapes and their statistics, for both binary and gray level images, is the granulometric function, also called granulometric size distribution (Ballarin and Valentinuzzi, 2001; Heijmans, 1991; Vincent and Dougherty, 1994).

The aim of this work is to develop algorithms to compute adequate indices for characterization and quantification of shape, angle and size of biological components, as well as the statistical distribution of these characteristics. These features are extremely variable in the images of material with different manufacturing condi-