

## Vibrational Characterization of Self-Assembling Oligopeptides for Tissue Engineering on TiO<sub>2</sub> Surfaces

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In the emerging field of tissue engineering, the development of synthetic materials promoting cells growth has led to the study of functionalised biomimetic materials and in particular to investigations on regular alternating polar/non-polar oligopeptides such as EAK-16, AEAEAKAKAEAEAKAK, first synthesised by Zhang et al. The characteristic of EAK-16 is to have a preferential  $\beta$ -sheet structure, to be resistant to proteolytic cleavage and to self-assemble into an insoluble macroscopic membrane. Its ability to create stable self-assembling layers derives from hydrophobic interactions between the  $-\text{CH}$  groups of non-ionic residues and complementary ionic bonds between acidic and basic amino acids: this stability can be enhanced by the regulation of pH and the presence of monovalent metallic ions. In order to evaluate the ability to form self-assembled layers on oxidised titanium surfaces, we investigated 7 different oligopeptides (16 to 19 residues) derived from EAK-16 but modified in their sequence by substitution of acid, basic and neutral amino acid or by the addition at the N-terminus of the RGD sequence, able to control osteoblast adhesion.

The techniques used to determine the structure of the oligopeptides were IR and Raman vibrational spectroscopies, which can provide useful information on the secondary structure of the peptides, both on the qualitative and the quantitative aspect, by the help of different amide stretching modes. The peptides were examined as synthesised and after deposition on oxidised titanium substrates.

A quantitative evaluation of the secondary structure of the oligopeptides was obtained by fitting the Raman amide I band. Almost all the peptides showed a preferential beta-sheet structure, as revealed by the position of the amide I band at 1670-1673  $\text{cm}^{-1}$  in their Raman spectra and at 1694-1697  $\text{cm}^{-1}$  and 1620-1626  $\text{cm}^{-1}$  in the IR ones. The substitution of the non polar amino acid (A $\rightarrow$  $\alpha$ -aminobutyrric acid and A $\rightarrow$ Y) induced in the first case an increase of alpha-helix conformation while in the second one allowed a higher order of the hydrophobic component. Mainly alpha-helix or mixed content was found in the peptides containing the RGD head, as revealed by the presence of Raman and IR bands at 1659 and 1640  $\text{cm}^{-1}$ , respectively.

Micro Raman and micro ATR/FT-IR techniques were used to study the conformational changes of the peptides deposited on oxidised porous titanium plates under physiological conditions (pH 7.4, in phosphate buffer). From a macroscopic point of view, some peptides formed a very ordered and homogeneous layer covering the entire metallic surface, while other peptides formed clusters of crystalline aggregates. However, vibrational analysis pointed out that all peptides, regardless of their macroscopic disposition on the surface, showed the typical amide I and  $\nu$  NH bands of beta-sheet. Another major feature of micro-IR and Raman spectra was the increase in intensity of the  $\nu_{\text{sym}} \text{COO}^-$  band at 1400  $\text{cm}^{-1}$ , indicating that the peptides interact with the surface by means of their carboxylic groups. The importance of  $\text{COO}^-$  was focussed by a further study with the SERS technique on silver colloids: the interaction with Ag particles is mediated by carboxylic groups and by aromatic groups when tyrosine is present in the primary structure of the peptides.