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Structural and rheological characterization of hyaluronic acid-based scaffolds for adipose tissue engineering

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Abstract

In this study the attention has been focused on the ester derivative of hyaluronic acid (HA), HYAFF®11, as a potential three-dimensional scaffold in adipose tissue engineering. Different HYAFF®11 sponges having different pore sizes, coated or not coated with HA, have been studied from a rheological and morphological point of view in order to correlate their structure to the macroscopic and degradation properties both *in vitro* and *in vivo*, using rat model. The *in vitro* results indicate that the HYAFF®11 sponges possess proper structural and mechanical properties to be used as scaffolds for adipose tissue engineering and, among all the analysed samples, uncoated HYAFF®11 large-pore sponges showed a longer lasting mechanical stability. From the *in vivo* results, it was observed that the elastic modulus of scaffolds seeded with preadipocytes, the biohybrid constructs, and explanted after 3 months of implantation in autologous rat model are over one order of magnitude higher than the corresponding values for the native tissue. These results could suggest that the implanted scaffolds can be invaded and populated by different cells, not only adipocytes, that can produce new matrix having different properties from that of adipose tissue.

Keywords: Adipose tissue engineering; Hyaluronic acid; Scaffold; Viscoelasticity

1. Introduction

Adipose tissue acts as a protective mechanical cushion for tissues and organs such as bones, vessels and nerves. Loss of this tissue can result from congenital malformations, HIV infections, complex traumatic wounds, extensive deep burns, pressure sores and oncologic resections, often causing considerable aesthetic and functional problems. No effective solution for the replacement of adipose tissue has been found. Autologous fat implant undergo shrinkage because of poor vascularization, whereas implants made of synthetic filling biomaterials might induce immune reaction and are rarely integrated by surrounding tissues [1–4]. Tissue engineering approaches offer considerable potential, but are clearly dependent on the development of appropriate scaffolds.

Recent studies focalized the attention on scaffolds made of synthetic and/or natural polymers, such as poly(lacto-*co*-glycolic) acid (PLGA) discs or injectable spheres [5,6], polytetrafluorethylene scaffolds [7], collagen gels [8], gelatine microspheres [9] or structures made by reinforcing poly(glycolic acid) fibre-based matrices with poly(L-lactic acid) [10]. Unfortunately, none of these approaches proved to be drawback-free and effective and none of them reached a proper developmental stage for clinical application in humans.

An ideal scaffold for adipose tissue engineering should provide specific biological stimuli, promote vascularization and possess the proper three-dimensional (3D) structure to allow adipose tissue cells to adhere, proliferate and reach

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a mature phenotype. Since adipocytes are voluminous cells, a spongy 3D structure is believed to be the most adequate scaffold for neo-adipogenesis starting from adipocyte precursors which need space for optimal differentiation. A high degree of porosity and extended specific surface of sponges, in addition, favour the permeability of large quantities of solutions and their homogeneous distribution, which are important features for any engineered construct. Moreover adequate mechanical properties are required for the scaffolds as it should have a sufficient strength to withstand *in vivo* stresses, protect cells from high pressure, being at the same time mechanically biocompatible with the native tissue. Proper scaffold degradation time is also desirable since it should not be resorbed too quickly after transplantation. With respect to scaffold chemical composition, natural derived polymers are believed to favour biocompatibility thanks to their cell interaction capability.

Among the natural materials hyaluronic acid (HA) has long attracted researchers' interest for its unique chemical–physical and biological properties. HA, indeed, is a high-molecular-weight polysaccharide that is a primary component of the extracellular matrix of the connective tissue which regulates and controls several tissue physiological functions *in vivo*. HA contributes to the viscoelastic properties of soft tissues and to their mechanical behaviour in compression [11], and it exerts important functions in joint lubrication, as well [12]. From a biological standpoint, the HA molecule plays a major role in tissue growth and remodelling as it specifically interacts with endogenous receptors such as CD 44, RHAMM, ICAM-1, regulating cellular migration, growth and adhesion [13]. However, the high water affinity strongly limits the use of the native molecule since it leads to poor mechanical properties. Furthermore, the use of unmodified HA as scaffold material for tissue engineering is severely hampered by its poor processability and handling properties. To circumvent these limitations, several approaches have been attempted to chemically modify HA by cross-link or coupling reactions, preserving at the same time its biological activity. A series of derivatives, called HYAFF®, has been obtained by the esterification of the carboxyl group of the glucuronic acid moiety of the polymer with linear or aromatic alcohol. Among these, the HA benzyl ester, HYAFF®11, has been widely used in the biomedical field thanks to its ease of processabilty and biocompatibility, which has been demonstrated both *in vitro* and *in vivo* [14,15].

HYAFF®11 has been clinically used in the form of non-woven felts and films for cartilage and skin tissue engineering [16–19]. HYAFF®11, in the form of sponges, was associated to human preadipocytes and the biohybrid constructs were proved to be effective in adipose tissue regeneration in a nude mouse model [20–22]. These studies highlight that scaffolds structure and architecture are key parameters in adipose tissue regeneration and in particular the pore dimension of the sponges was suggested to be crucial for an extensive adipose tissue differentiation in the whole section of the construct.

In this context, the aim of this work was to perform a systematic characterization, by *in vitro* and *in vivo* testing, of different HYAFF®11 spongy scaffolds potentially useful in adipose tissue engineering. The scaffold properties were studied by SEM analysis, water absorption tests and rheological characterization. The degradation properties were investigated *in vitro* by monitoring the rheological behaviour and morphological changes as a function of incubation time in cell culture medium; while, *in vivo*, by monitoring the rheological properties of the explanted biohybrid constructs as a function of the implantation times in autologous rat models. The *in vivo* studies play a key role in the understanding of the cell ability to degrade the scaffolds and to produce an extracellular matrix which is mechanically biocompatible with the native tissue.

2. Experimental

2.1. Materials

2.1.1. HYAFF®11 sponges

HYAFF®11 is a linear derivative of HA obtained by total esterification (>90%) of the carboxyl groups with benzyl alcohol, as previously described [19]. Different HYAFF®11 sponge have been supplied by Fidia Advanced Biopolymer (FAB). In particular, sponges with "small" and "large" pore size were obtained using inorganic salts of granulometry of 200–315 and 400–500 μm, respectively. A hydrophilic HA coating was added to the scaffold structure with the main aim to facilitate the process of cell seeding, through a fast medium absorption. HYAFF®11-coated sponges were obtained by immersion of HYAFF®11 sponges in an aqueous solution of HA biopolymer at a known concentration, and were subsequently freeze-dried. Scaffolds were sterilized by gamma irradiation at 25 Kgy. Sponges produced for this study were characterized by less than 0.2 EU/mg endotoxins and less than 0.1%w/w residual solvents (free benzyl alcohol, ethanol, acetone, DMSO, chloride). HYAFF®11 sponges used in this study are summarized in Table 1.

Table 1
Description of HYAFF®11 sponges used in this study and their water absorption properties

HYAFF11 sponge type	Granulometry of the salt used in the production process (μm)	Acronym	Water absorption (w/w%)
Small pore	200–315	HY	380±38
Small pore, HA coated	200–315	HYB	1362±15
Large pore	400–500	HYLP	806±140
Large pore, HA coated	400–500	HYBLP	1517±80

Data are means of % absorption of three sponge batches of each type.