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Leading Opinion

Inflammatory signals in the development of tissue-engineered soft tissue ☆

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Abstract

Scaffolds with 400 μm pores constructed from hyaluronan modified by benzyl esterification of the carboxylic acid groups (HYAFF-11) and viscous gels created from dodecyl-amidation of hyaluronan (HYADD-3) were implanted subcutaneously into rats for periods of up to 26 and 12 weeks, respectively. Tissue explants were infiltrated with methacrylate resin, sectioned and stained with a broad panel of inflammatory markers in addition to conventional histological stains. Both gels and sponges became rapidly infiltrated by cells that, in the case of HYAFF sponges, did not differentiate, whilst mature adipocytes were only observed at the margins of the sponges. This was combined with sustained inflammatory antigen expression. Conversely, in the HYADD gels, only moderate inflammatory staining was observed at 4 weeks which had diminished completely by 8 weeks. Mature and maturing adipocytes were observed deep within the gels. It is hypothesised that the gels present an excellent inflammatory cytokine profile which induces macrophage infiltration, proliferation then differentiation into adipocytes and is responsible for the generation of neoadipogenesis.

Keywords: Soft tissue; Inflammation; Macrophage; Adipocyte; Tissue engineering

1. Introduction

Deficiencies and traumatic loss of soft tissue due to congenital disorders [1,2], disease or injury has a major debilitating effect on patients due to increased friction generated between the dermis and bone. High levels of movement, necessary for the articulation of joints and muscles under the skin, can be generated in normal soft tissue due to the presence of a layer of white adipose tissue in the subcutaneous layer. The normal clinical treatment for these conditions is transplantation of mature, autologous fat tissue or free fat transplantation [3,4], which has an unreliable outcome ranging from complete resorption, gross shrinkage of the graft and the formation of oily cysts. Common fillers of small defects, such as collagen, native hyaluronic acid or synthetic polymers such as PMMA and silicone, generally have short subcutaneous residence times and can cause a chronic host response [5–8]. Clinical treatments that require large volumes of adipose tissue, such as breast reconstruction after mastectomy, are often difficult to perform satisfactorily on slim patients.

2. Tissue engineering solutions

Tissue engineering would appear to be an obvious solution to the problem of adipose tissue transplantation efficacy and availability. A common paradigm would involve the implantation of a scaffold seeded with autologously derived progenitor cells. Recent studies have utilised synthetic polymer scaffolds such as PGA [9], PLGA [10] and PTFE [11], but these have been shown to have severe limitations, such as mechanical matching for application in adipose tissue. Many research studies have

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used Matrigel [12,13] to demonstrate preadipocyte conversion into mature adipose tissue, but this is unusable in real clinical situations. Perhaps the most promising results have been achieved with naturally derived scaffolds based on polymers such as collagen [14,15] and hyaluronan [16]. A study of the literature to date has provided little evidence of the long-term efficacy of tissue engineering strategies of adipose tissue for clinical applications, however.

3. Fat tissue

The clinical application of tissue and organ transplantation has been continuously refined over many years since the first successful corneal transplant in 1905 by Eduard Zirm and the first living kidney transplant in 1954 by Joseph Murray and David Hume. Today, many different tissues and organs are successfully transplanted from both live and dead allogeneic donors, yet procedures for autologous fat transplantation have hardly changed over this time frame, and is rarely truly successful [3]. Transplanted tissues quickly connect their vascular systems to the host blood supply, limiting tissue necrosis. Fat tissue is highly vascularised, and it is hypothesised that the inadequate connection of this vasculature to the host is responsible for the tissue experiencing severe ischaemic conditions [17], particularly in the case of free fat transplantation, resulting in gross adipocyte necrosis.

Excess fat, present in a significant proportion of the population, is rarely appreciated yet white adipose performs a vital mechanical role. In the recent past, it was considered to be waste material of little biological interest. Recently, however, adipose tissue is emerging as an important organ in its own right, delivering vital inflammatory and immune functions, releasing TNFα, IL-6 and leptin [18,19]. Furthermore, obesity has begun to be viewed as a chronic low-grade inflammatory condition [20–22]. The apparently simple proposition of recreating a simple, oily, collection of cells with little structured matrix should now be reconsidered as the reconstruction of a complex inflammatory and immune system.

Only recently has the presence of lymphoid cells in natural adipose tissue been reported in the literature [23], their phenotype suggesting more innate rather than adaptive immune roles [24]. Furthermore, further evidence for the inflammatory role of adipose tissue was provided by the observation of macrophage–adipocyte interconversion [25]. The whole basis of the reported inflammatory conditions within model engineered-adipocyte studies may need to be revisited, as observations in two recent studies demonstrate [26,27], discussed below.

4. Experimental engineered adipose

4.1. Solid scaffolds in vivo

An *in vivo* study of adipose regeneration was conducted using scaffolds 15 mm in diameter and 5 mm thick [26]. Sponges based on a well-known hyaluronan derivative, HYAFF-11 [28], in which the carboxylate groups had been benzyl-esterified to create a solid material, were created with 400 μm pores by salt leaching, and donated by Fidia Advanced Biopolymers (Abano Terme, Italy). These were implanted, in acellular form, subcutaneously into rats for periods of up to 26 weeks, explanted, fixed into methacrylate blocks and stained for a wide panel of inflammatory and lymphoid antigens in addition to conventional histological stains. Hyaluronic acid-based scaffolds were chosen because the monomeric hyaluronan degradation products are known to be highly angiogenic, a condition often associated with adipose tissue development [29].

Initial observations of histological sections showed cellular infiltration, appearing to be a typical macrophage response, sustained over several months, with a gradual accumulation of adipocytes at the periphery of the scaffold (Fig. 1). Although angiogenesis could be observed from between 4 and 8 weeks after implantation, no concomitant formation of adipose tissue was apparent within the

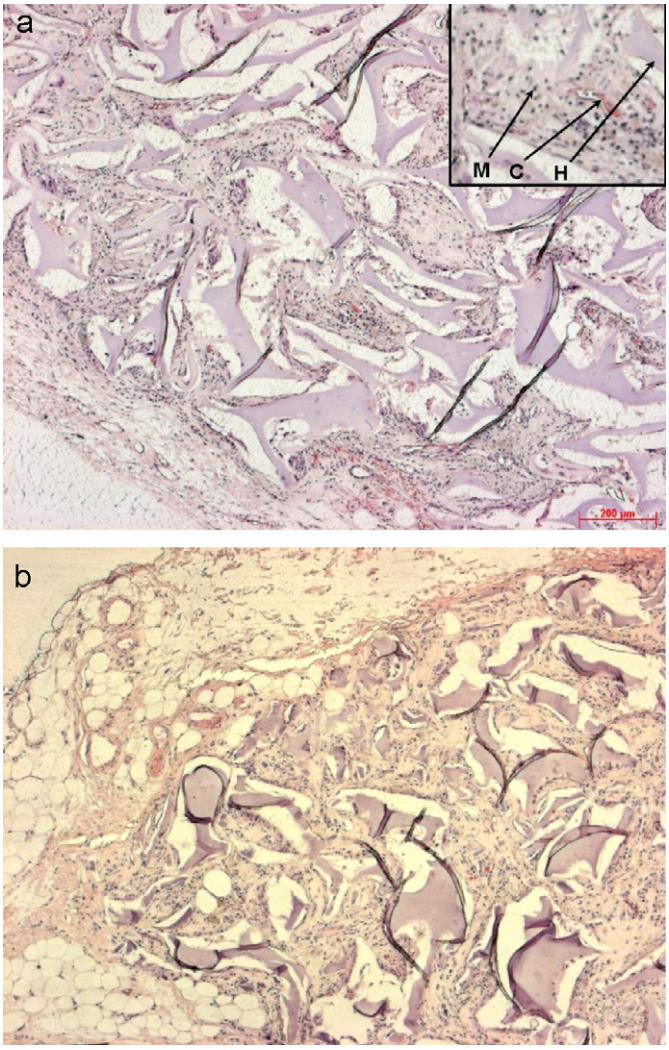

Fig. 1. Acellular HYAFF-11 scaffold implanted subcutaneously into rats, for (a) 8 weeks; (b) 12 weeks; stained by haematoxylin and eosin; M—macrophage; C—capillary; H—HYAFF.

scaffolds. There was expression of TGFβ and MHC-II antigens within the scaffold at all time periods (Fig. 2), suggesting a sustained inflammatory response that was not likely to result in the formation of adipose tissue.

Interestingly, further analysis of the sections with stains for vimentin expression and macrophage differentiation indicated high levels of both antigens at 26 weeks, whereas both were more weekly expressed at earlier time periods. It has been observed that vimentin expression is associated with both human and rat differentiating preadipocytes [30]. The expression of macrophage differentiation marker expressed on a similar proportion of macrophages to that of cells expressing vimentin suggests that preadipocyte conversion into adipocytes may be occurring. This would be consistent with previous descriptions of reduced adipocyte differentiation in inflammatory milieu [31,32]. On the contrary, inflammatory chemokines induce the infiltration of both preadipocytes and circulating cells which have the capacity to differentiate into adipocytes [33,34]. Additionally, TGFβ, expressed in small quantities in the scaffolds, has been described as a promoter of preadipocyte proliferation [35], but inhibitor of differentiation [36]. This may suggest that the initial inflammatory host response may initiate the infiltration response, but the conversion into adipocytes, as demonstrated by Saillan-Barreau et al. [25], is suspended until the initial response recedes. The timing of this macrophage-like preadipocyte conversion, after more than 6 months, is likely to be too protracted for efficacious clinical use.

4.2. Gel matrices in vivo

A novel gel based on hyaluronan, but amidated with 12 carbon amide chains (HYADD-3), was prepared by Fidia Advanced Biopolymers (Abano Terme, Italy) and implanted in the same manner as the HYAFF-11 sponges in

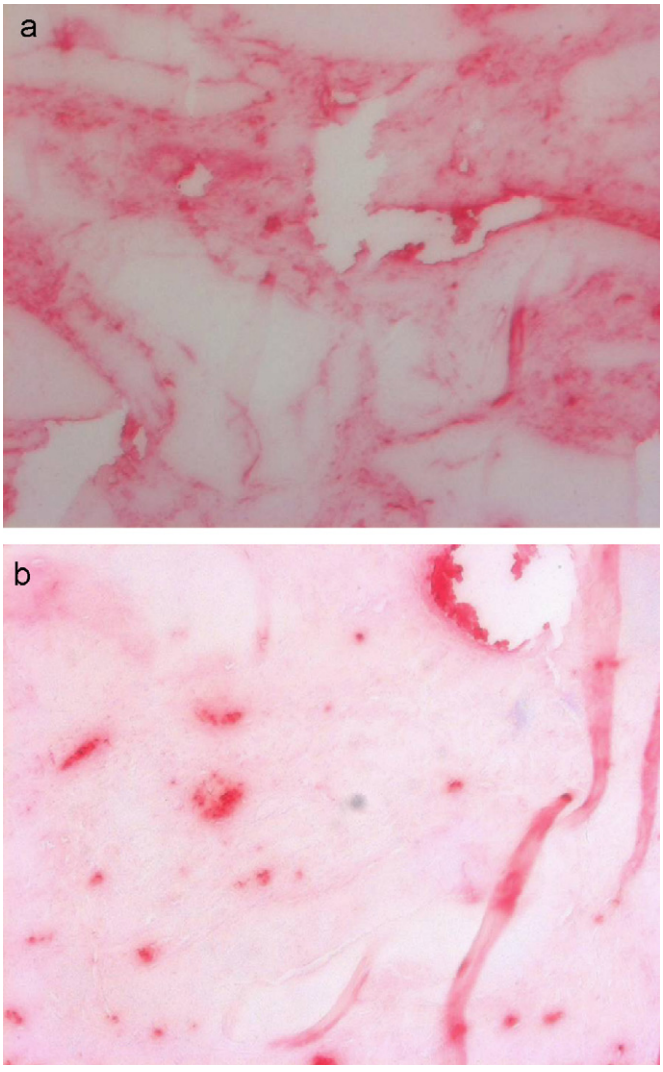

Fig. 2. Acellular HYAFF-11 scaffold implanted subcutaneously into rats for 26 weeks, not counterstained, immunostained for (a) MHC-I; (b) TGFβ [negative control is blank].

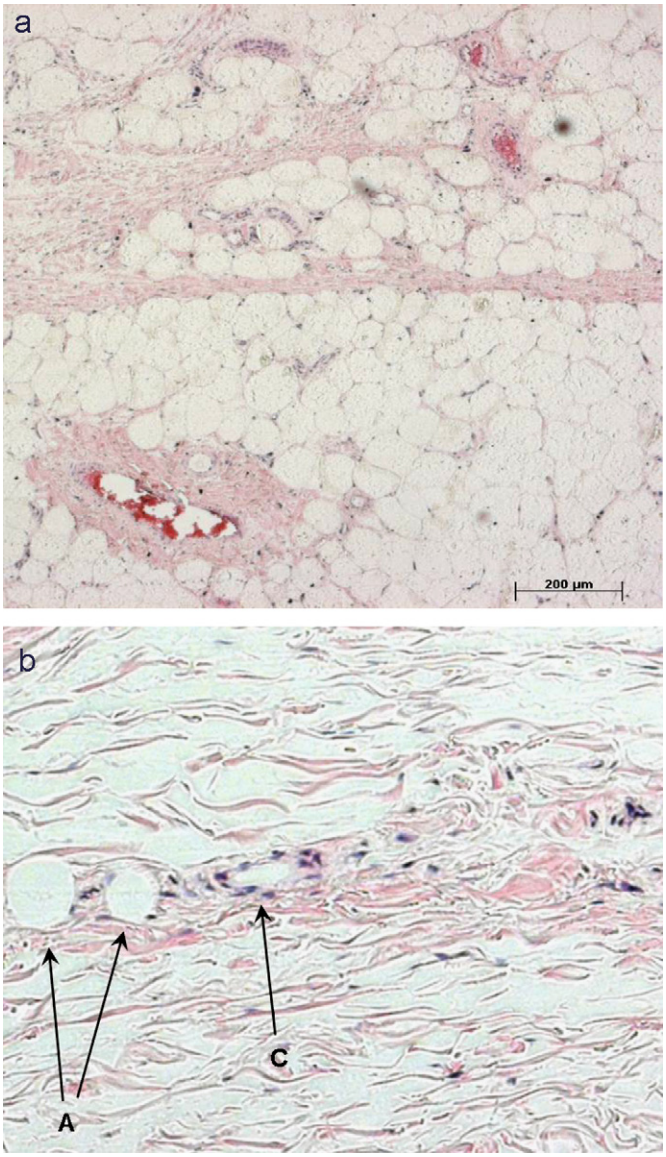

Fig. 3. HYADD-3 implanted subcutaneously into rats for 12 weeks; stained by haematoxylin and eosin; A—adipocyte; C—capillary.

acellular form, but for periods of up to 12 weeks [27]. Histological analysis of the 1-week samples demonstrated very little cell infiltration, although this was greater after the 2–4-week period. Significant angiogenesis was observed and the presence of mature and maturing adipocytes seen deep within the gel (Fig. 3). The response to HYADD-3 was completely different to HYAFF-11. Whilst the matrix presented a more physiological form with respect to its mechanical properties, the gel degradation products were predicted to be similar to the esterified sponges.

The nature of the resultant inflammatory response was completely different in the HYADD-3 gel. There was intense vimentin staining at only 4 weeks (Fig. 4) combined with strong differentiating macrophage staining. There was only moderate inflammatory antigen staining (e.g. MHC-I, MHC-II, CD54) at 4 weeks, but this had declined to practically undetectable levels by 8 weeks (Fig. 5).

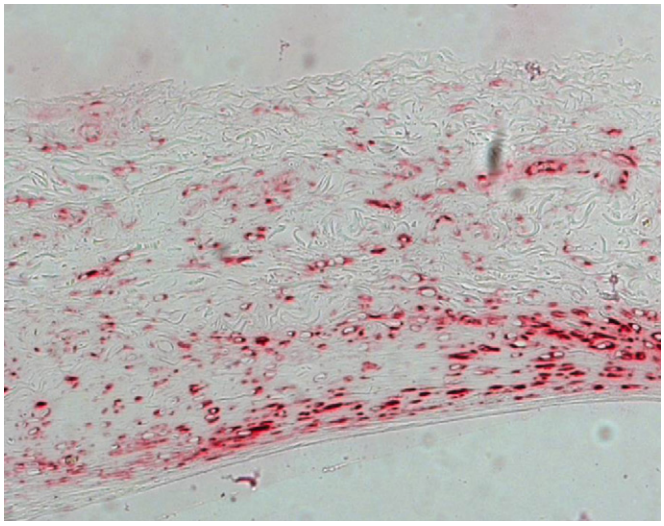

Fig. 4. HYADD-3 implanted subcutaneously into rats for 4 weeks; not counterstained, immunostained for vimentin [negative control is blank].

5. Concluding remarks

The conventional interpretation of large numbers of macrophages infiltrating within porous biomaterials implanted subcutaneously would be the initiation of an inflammatory response. Typically, macrophages would be activated to produce a spectrum of inflammatory cytokines and enhance the biomaterial degradation by due to phagacytosis. The presence of a fibrous capsule can be indicative of, depending on the desired application, lack of biocompatibility. Whilst both materials described in these studies did not produce a fibrous capsule, both produced what appeared to be, on first inspection, a conventional inflammatory infiltration. The recent observation that macrophages readily undergo transdifferentiation into adipocytes [25] may change this interpretation. Instead, macrophages, already known as drivers of tissue remodelling after injury, would be intrinsic to the formation of

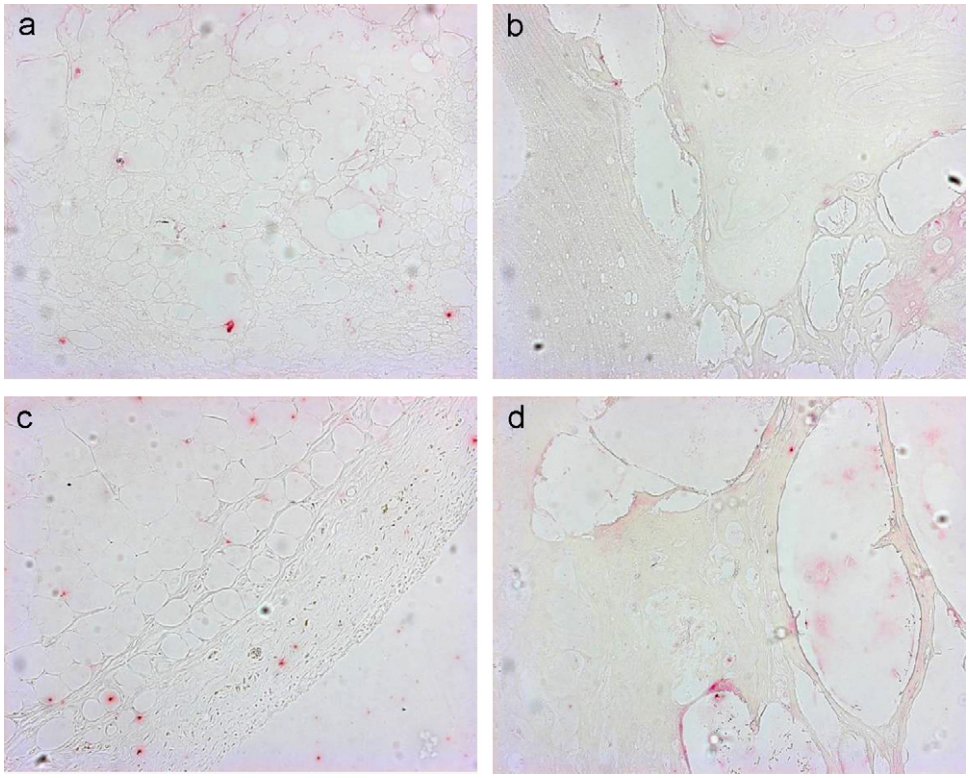

Fig. 5. HYADD-3 implanted subcutaneously into rats for 12 weeks; not counterstained, immunostained for (a) MHC-I; (b) MHC-II; (c) CD54; (d) TGFβ [negative control is blank].

de novo adipose tissue. This could explain why macrophages observed in the HYADD gels after initial time points, are accompanied by mature adipocytes during the latter stages.

The results in this study may be interpreted as a demonstration of an inflammatory cytokine profile for the generation of neo-adipose tissue. It is hypothesised that the inflammatory cytokine signalling was not intense, but great enough to induce the infiltration of macrophages in the gel. As the host inflammatory declined after 4 weeks, the conditions were correct for the differentiation of the macrophages into adipocytes, possibly following proliferation under the action of TGFβ, influenced by the angiogenic action of the degrading gels.

Whilst further studies will need to be performed to establish the precise nature of any possible transdifferentiation of macrophages in hyaluronan-based materials, current evidence of the interconnection between the inflammatory, immune and adipose systems suggest that closer scrutiny of the interpretation of the host response to tissue engineering scaffolds is required.

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