CHAPTER IV

RESULTS

4.1 Human Acellular Dermis (hADM) and Human de-epidermized Dermis (hDED)

4.1.1 Gross appearance of hADM and hDED

Comparing the morphological properties between hADM and hDED, we found that hADM tended to be softer and looser structure than hDED. hADM also had better swelling property than hDED.

In generally, as shown in Figure 4.1 and 4.2, color and others morphological structures of the product were quite similar with the commercial product (Surederm®).

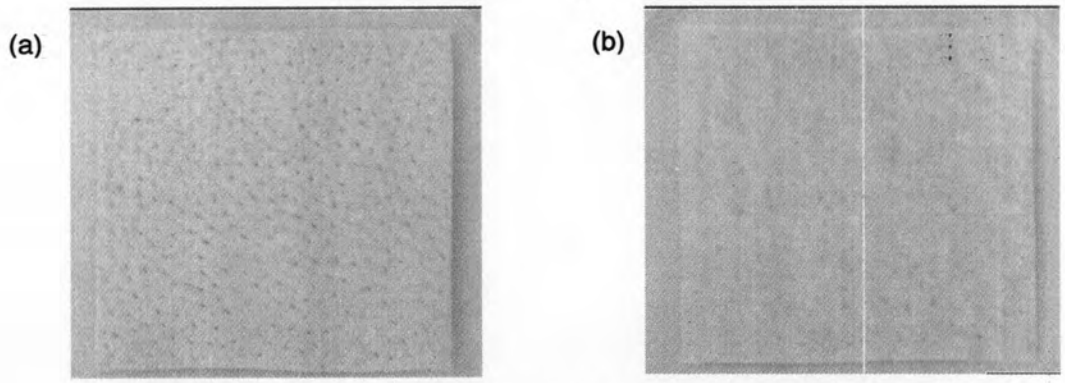


Figure 4.1: Pictures of human acellular dermis, papillary dermis (a) reticular dermis (b)

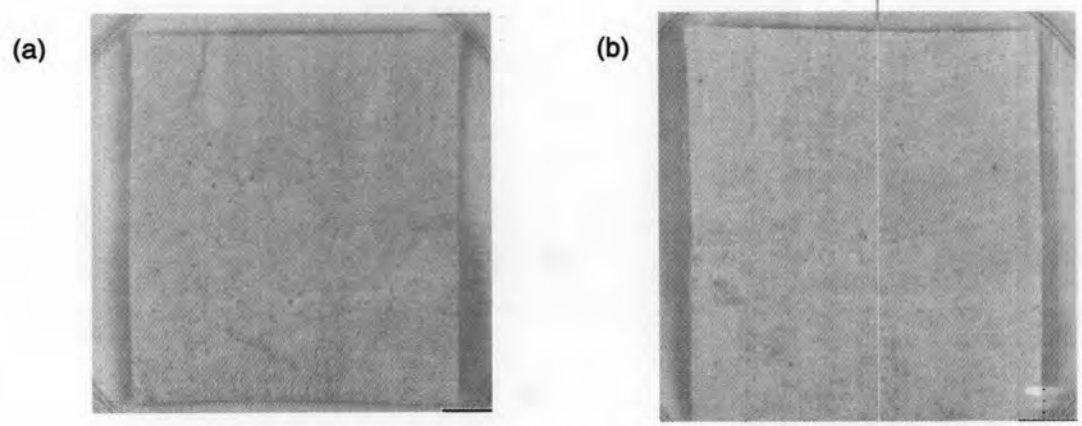
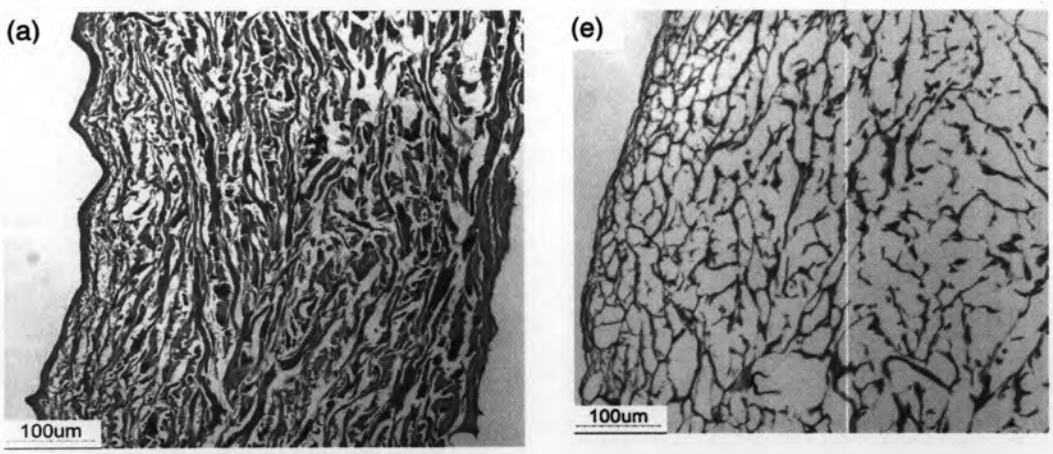


Figure 4.2: Pictures of human de-epidermized dermis, papillary dermis (a) reticular dermis (b)

4.1.2 H&E staining and immunohistrochemistry of hADM and hDED

As evident in Figure 4.3 of H&E staining, collagen networks were appeared in pink color. In hADM, collagen networks looked looser, more recticute, and shorter fragment than hDED consensus with the result of fluorescence staining shown in Figure 4.4. However, in Figure 4.3a-b hDED revealed the dense and large collagen network. Fibroblast cells had still been found in both hDED and hADM but there were more cells left in hDED than hADM as shown in Figure 4.3c-d.



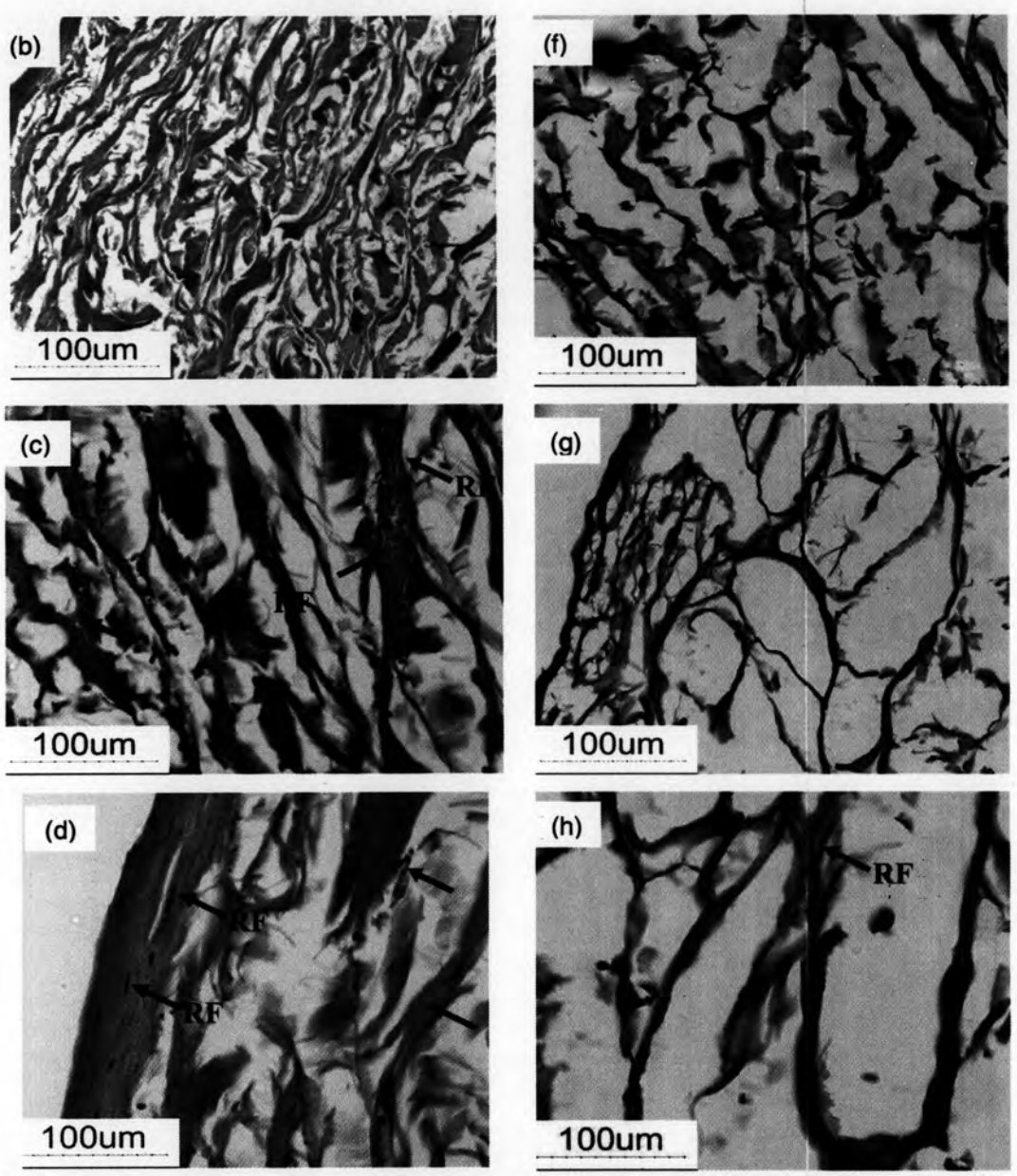


Figure 4.3 H&E staining under light microscope of hDED 40x (a), 100x (b), 200x (c-d) and hADM 40x (e), 100x (d-e), 400x (h)

Remnant fibroblasts in collagen bundles (RF)

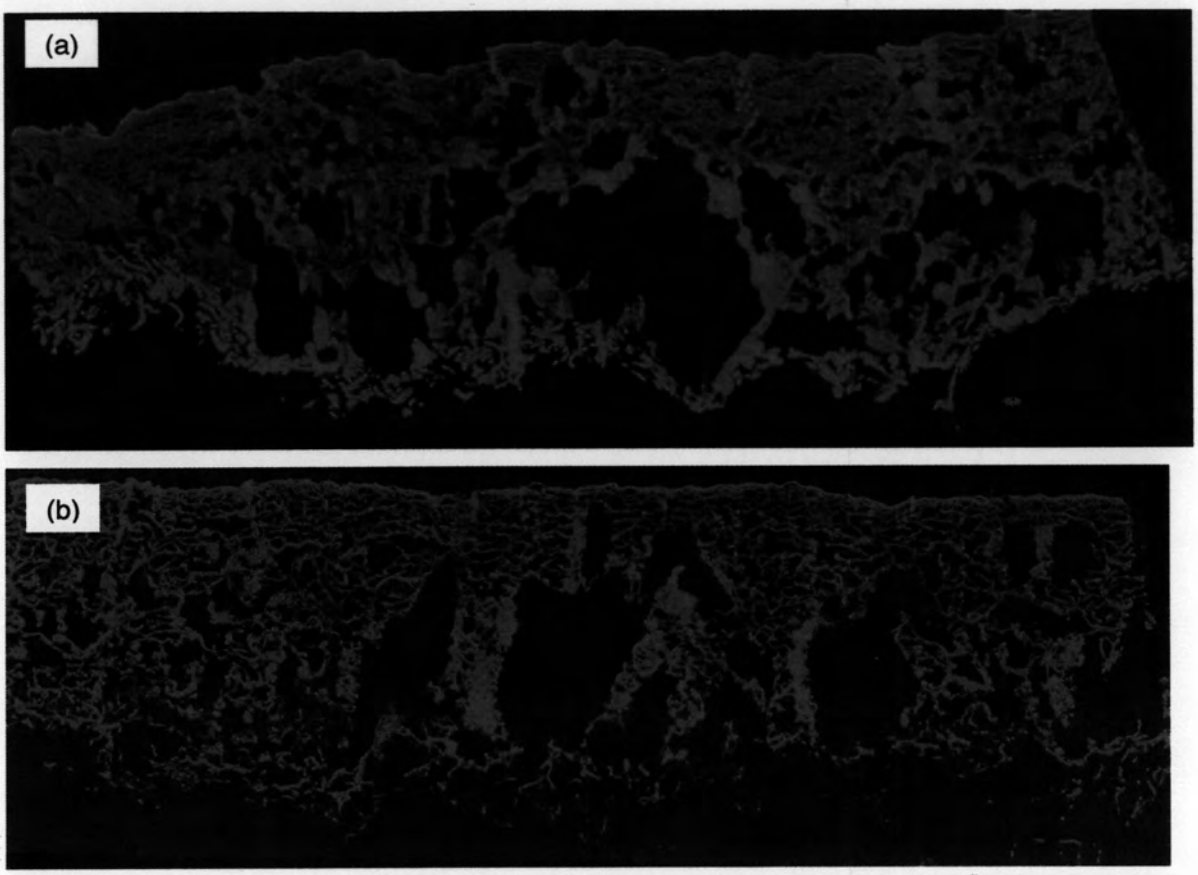


Figure 4.4 Confocal microscopic pictures compared of center section of specimens between hDED (a) and hADM (b) (100x)

4.2 Animal study (*In vivo* study)

4.2.1 The 1st week of *in vivo* study

In gross specimens, as illustrated in Figure 4.5 (a-e), hDEDs have a better response than hADMs (Figure 4.5 f-j). We found numerous neo-capillaries generate around the specimens. In the other hand, there were almost no responses in hADM. There were slight neo-capillaries generated around but no invasion into the specimens.

After the specimens were cut and stained by H&E staining, in hDED group, we can found host fibroblast cells infiltrated into the specimens and differentiated themselves into fusiform structure (Figure 4.7b.) and deposited new extracellular matrix, as illustrated in Figure 4.7a. Moreover, neo-capillaries were also investigated as shown in Figure 4.7b-c and we rarely found host rejected reaction occurring in hDED specimens. There were fusiform fibroblast cells attached with the old collagen fibers. This indicated that hDED was the suitable scaffold for fibroblast cells. In contrast, in hADM group, we found severe foreign body reaction in Figure 4.7d. There were numerous lymphocytes attached with the old collagen fibers as shown in Figure 4.7f. None of neo-collagen or new extracellular matrix was found in the specimens in Figure 4.7e.

In fluorescent staining pictures, hDED in the 1st week tended to be more collagen than that picture of hDED before implanted as shown in Figure 4.9a-b related with H&E staining of the 1st week hDED specimens above. On contrary, hADM were still more fibrous structure. As illustrated in Figure 4.9d-e, indicated that there weren't any neo-collagen deposited after 1'st week of implantation.

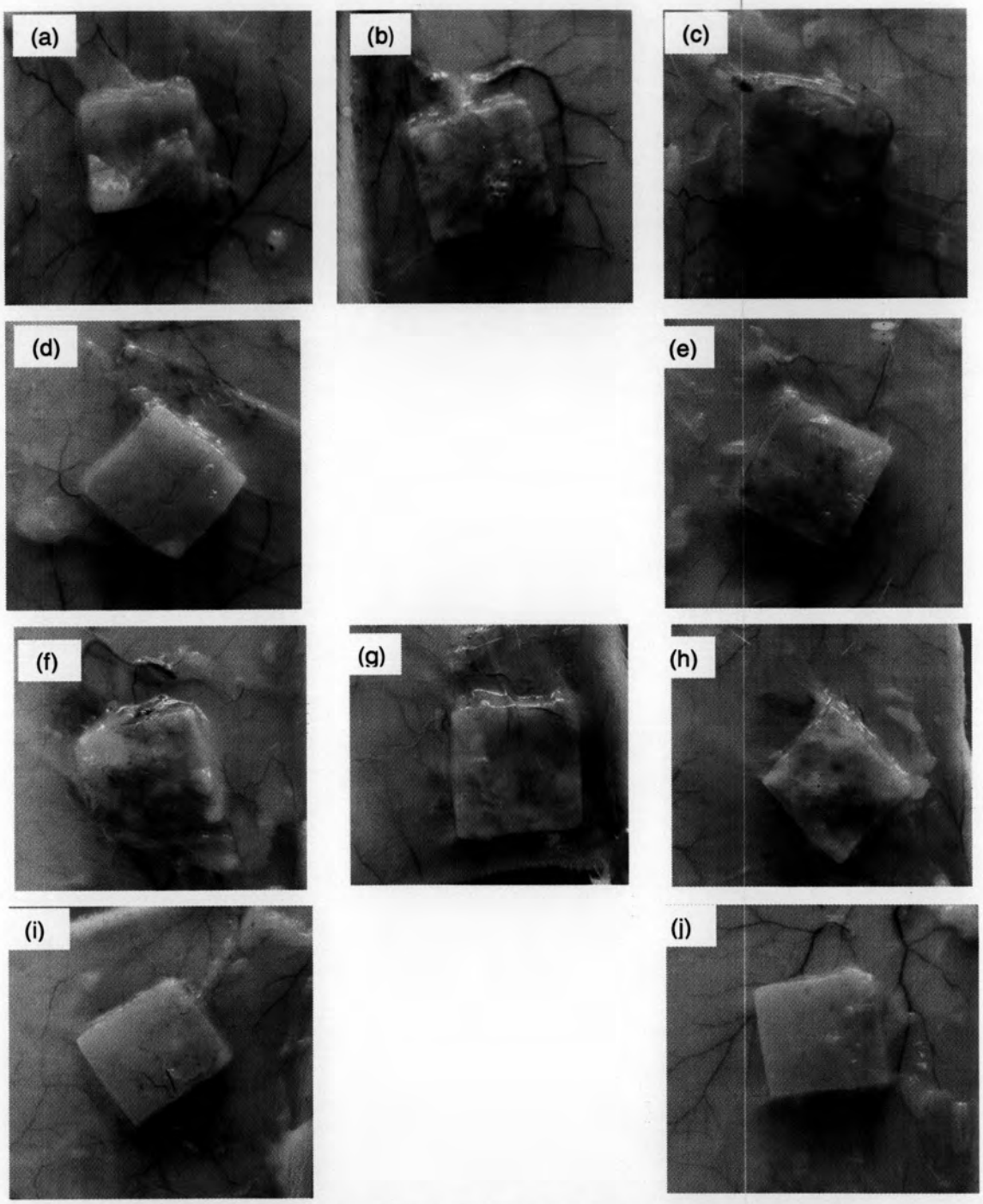


Figure 4.5 Gross specimens of (n=5) hDED (a-e) and hADM (f-j) of *in vivo* specimens at 1st week

Figure 4.6 Comparison of center section between hDED (a) and hADM (b) of *in vivo* specimens H&E staining section at 1st week (40x)

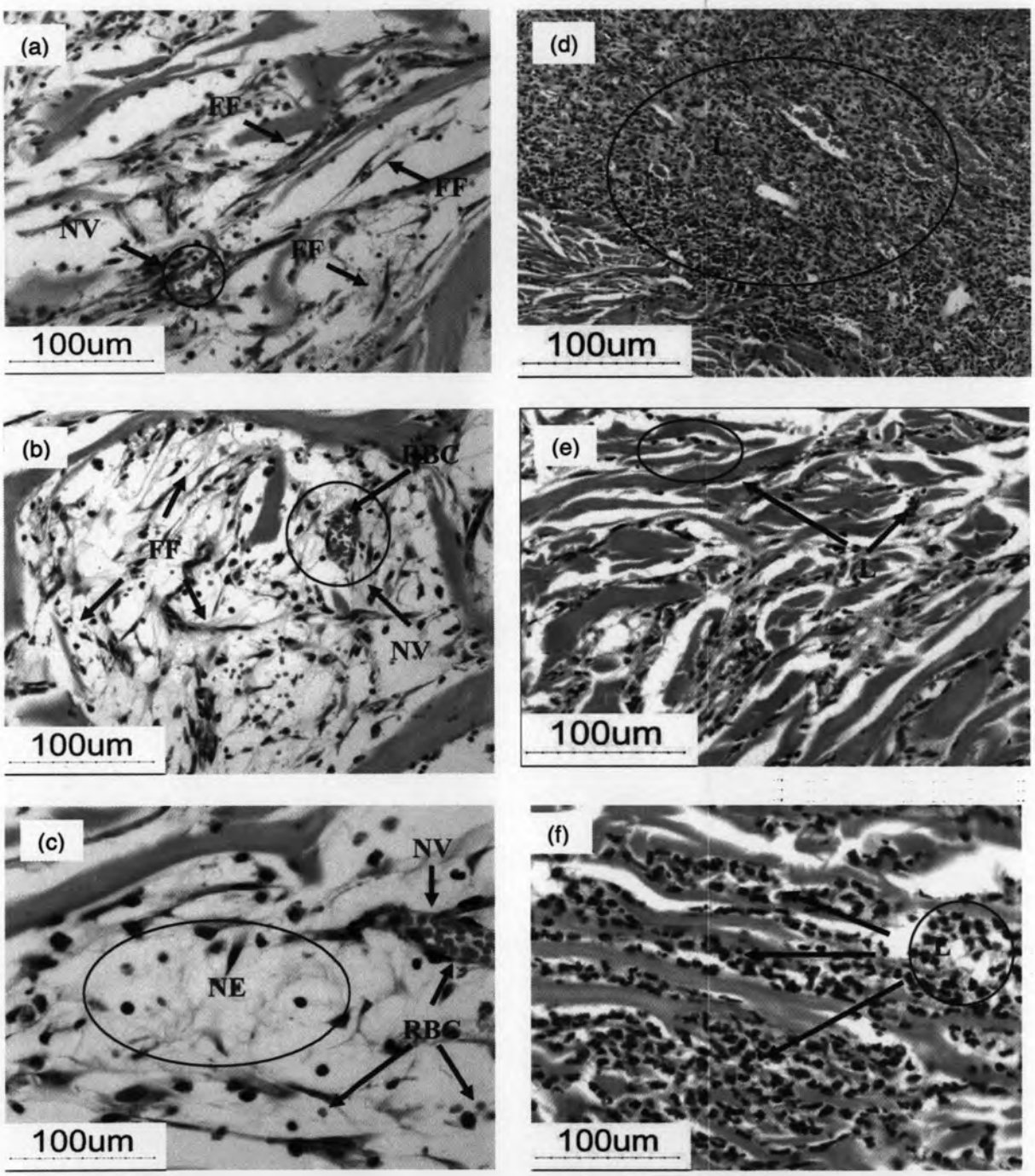


Figure 4.7 H&E staining under light microscope of hDED 100x(a), 200x (b), 400x (c) and hADM 100x(d), 200x (e),400x (f) of *in vivo* specimens H&E staining section at 1st week Fusiform fibroblast (FF), Neo-vascular (NV), Neo-extracellular matrix (NE), Lymphocyte (L), Red blood cell (RBC)

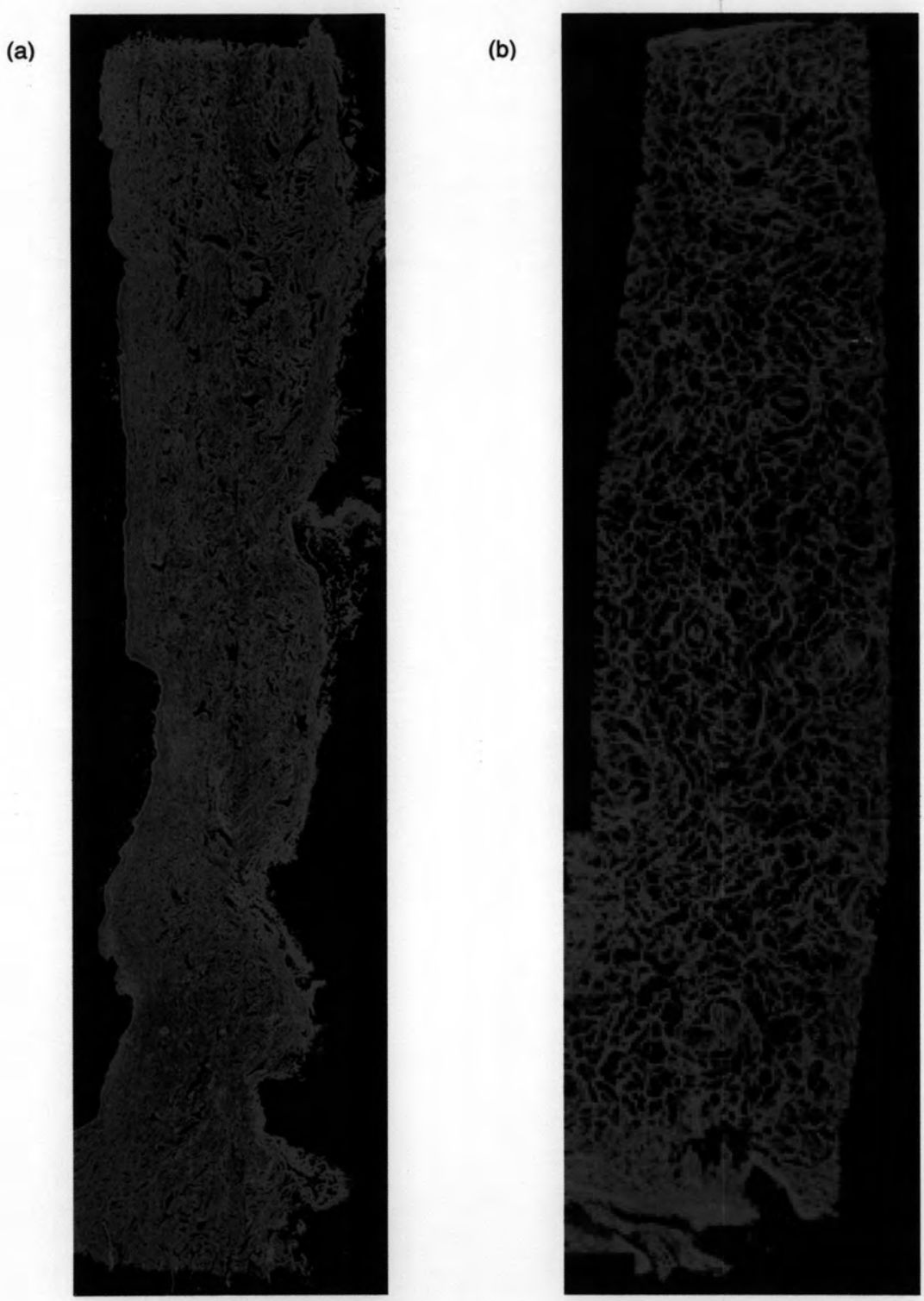


Figure 4.8 Confocal microscopic pictures compared of center section of *in vivo* specimens between hDED (a) and hADM (b) at 1st week (100x)

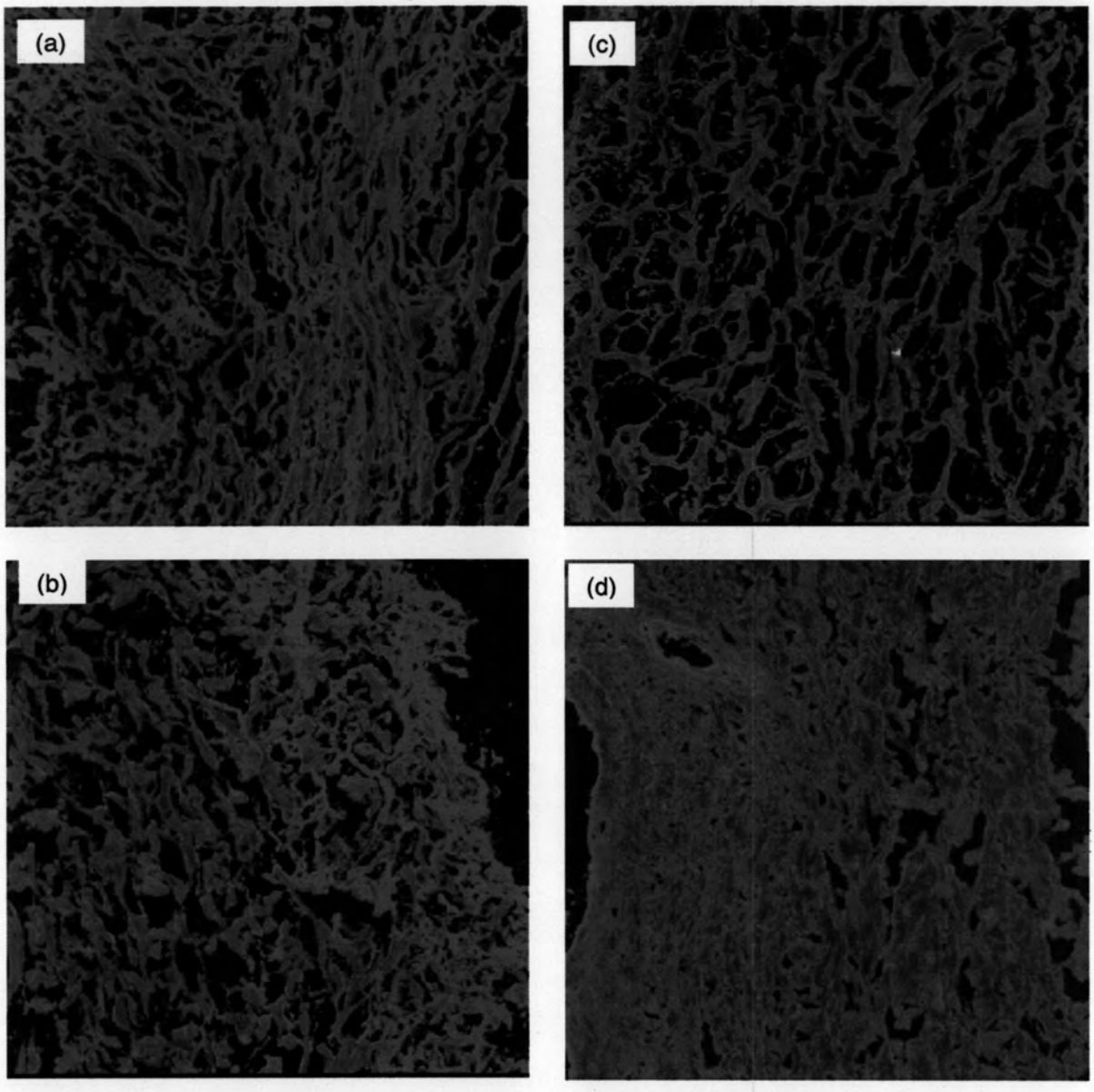


Figure 4.9 Confocal microscopic pictures compared of edge section of *in vivo* specimens between hDED (a-b) and hADM (c-d) at 1st week (100x)

4.2.2 The 2nd week of *in vivo* study

As illustrated in Figure 4.10a-c, we can still found that hDED specimens were in red but slightly decreased in concentration. However, hDED were still more host-response reaction than hADM which started to have host-response as shown in Figure 4.10f. There was slight integration of a specimen and host tissue as shown in Figure 4.10a. Though neo-vascularizations around the specimens were found, there weren't any evident of angiogenesis inside the specimens Figure 4.10d-e.

In H&E staining result, figure 4.11b significantly shown the foreign body reaction which a number of lymphocytes gathered around and infiltrated into the specimens of hADM. In the other hand, as shown in Figure 4.11a, in stead of foreign body reaction, neo-vascularization and neo-collagen were easily seen. If we watched closely under light microscope, as shown in Figure 4.12d-e, it noticed that a large amount of lymphocytes gathered along the host-collagen fibers. Besides that there weren't any new extracellular matrix formed between collagen fibers in Figure 4.12 f. In Figure 4.12a-b denser neo-collagen formation between host-collagen fibers was seen as light pink stained. Finally, Figure 4.12c had shown suitable form of differentiated fibroblasts and host-collagen attached fibroblasts provided new extracellular matrix

As a result of fluorescent staining shown in Figure 4.13b we can see that there were fibrous tissue occurred between the spaces if we compared with 1st week hADM specimens. In Figure 4.14a-b, the collagen structure seemed like woven-networks because there were a number of spaces between the fibers. However, in Figure 4.14d-e, related with H&E staining of hADM, the collagen structure looked like compacted and patched tissue in stead of woven-network.

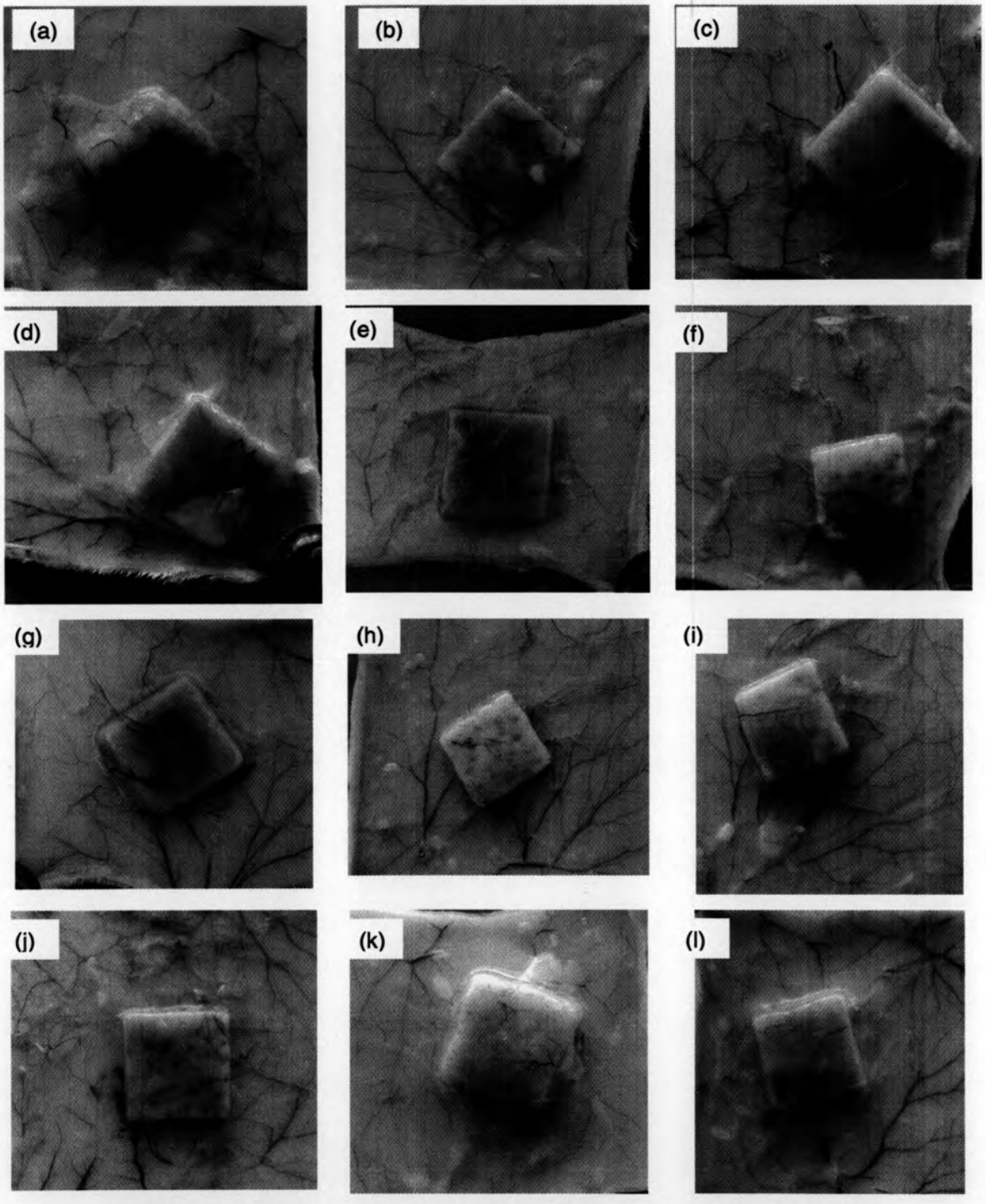


Figure 4.10 Gross specimens (n=6) of hDED (a-f) and hADM (g-l) of *in vivo* specimens at 2nd week.

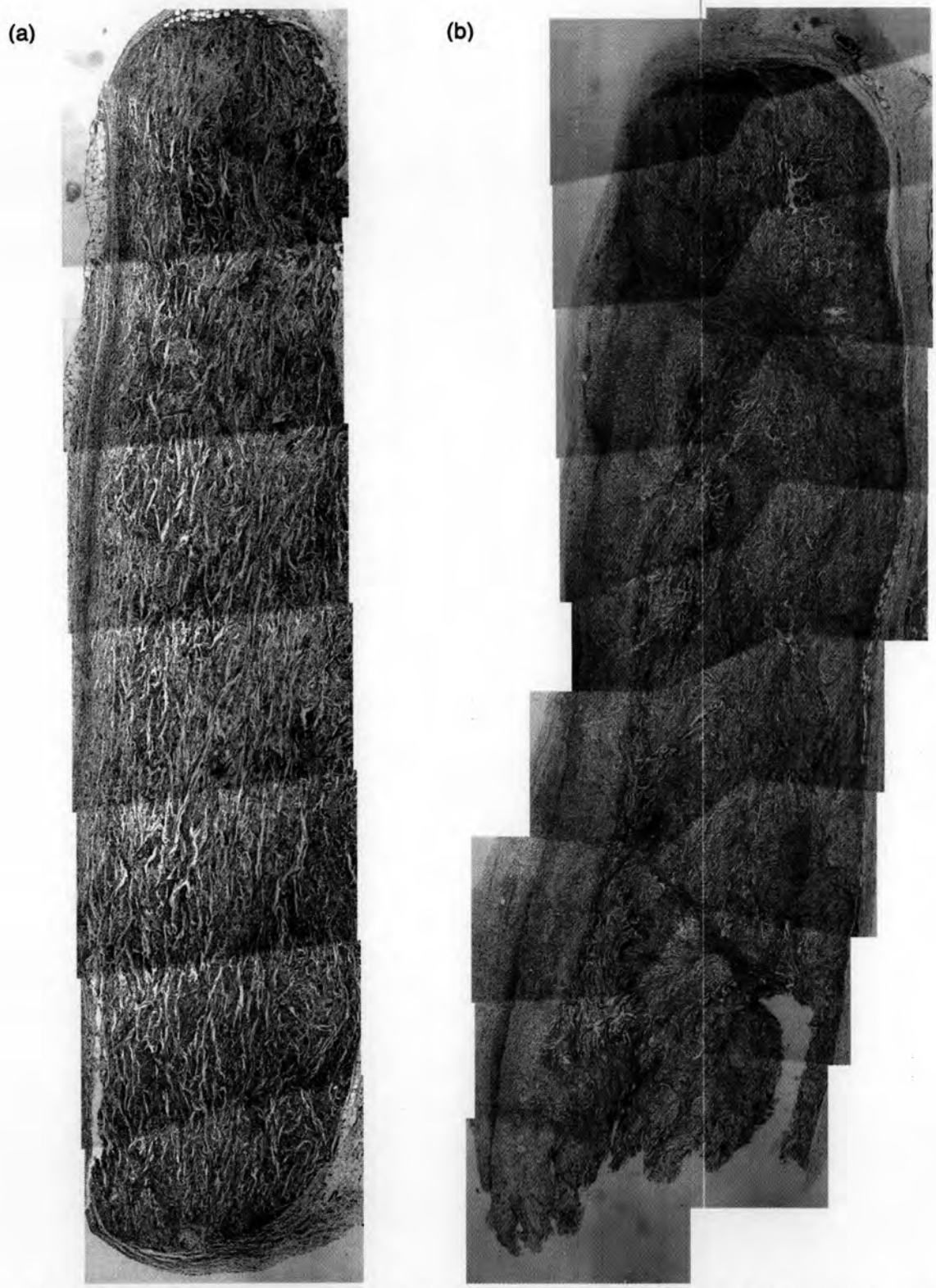


Figure 4.11 Comparison of center section between hDED (a) and hADM (b) of *in vivo* specimens H&E staining section at 2nd week (40x)

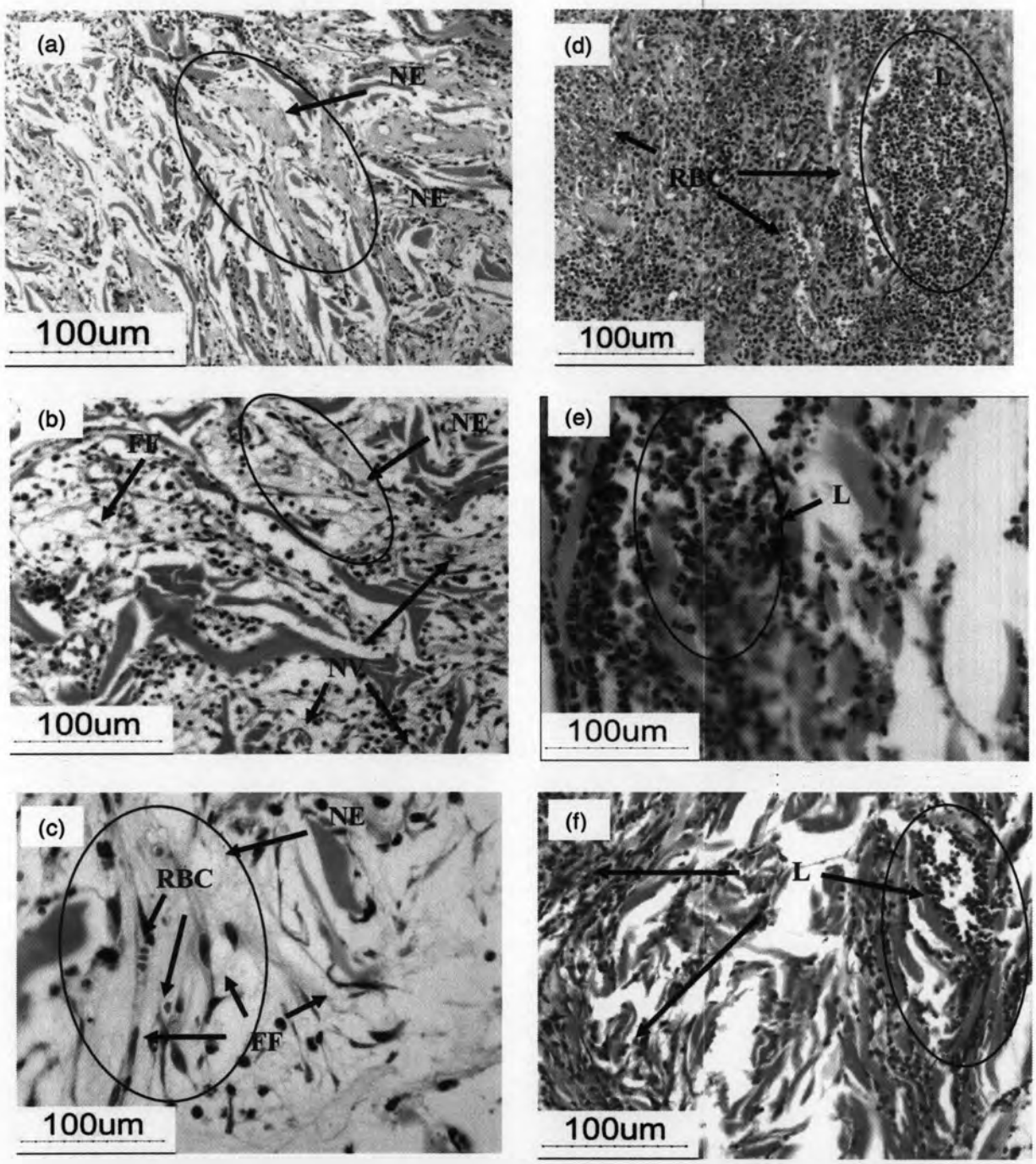


Figure 4.12 H&E staining under light microscope of hADM 100x(a-b), 400x (c) and hDED 100x (d), 200x (e), 400x (f) of *in vivo* specimens H&E staining section at 2nd week Fusiform fibroblast (FF), Neo-vascular (NV), Neo-extracellular matrix (NE), Lymphocyte (L), Red blood cell (RBC)

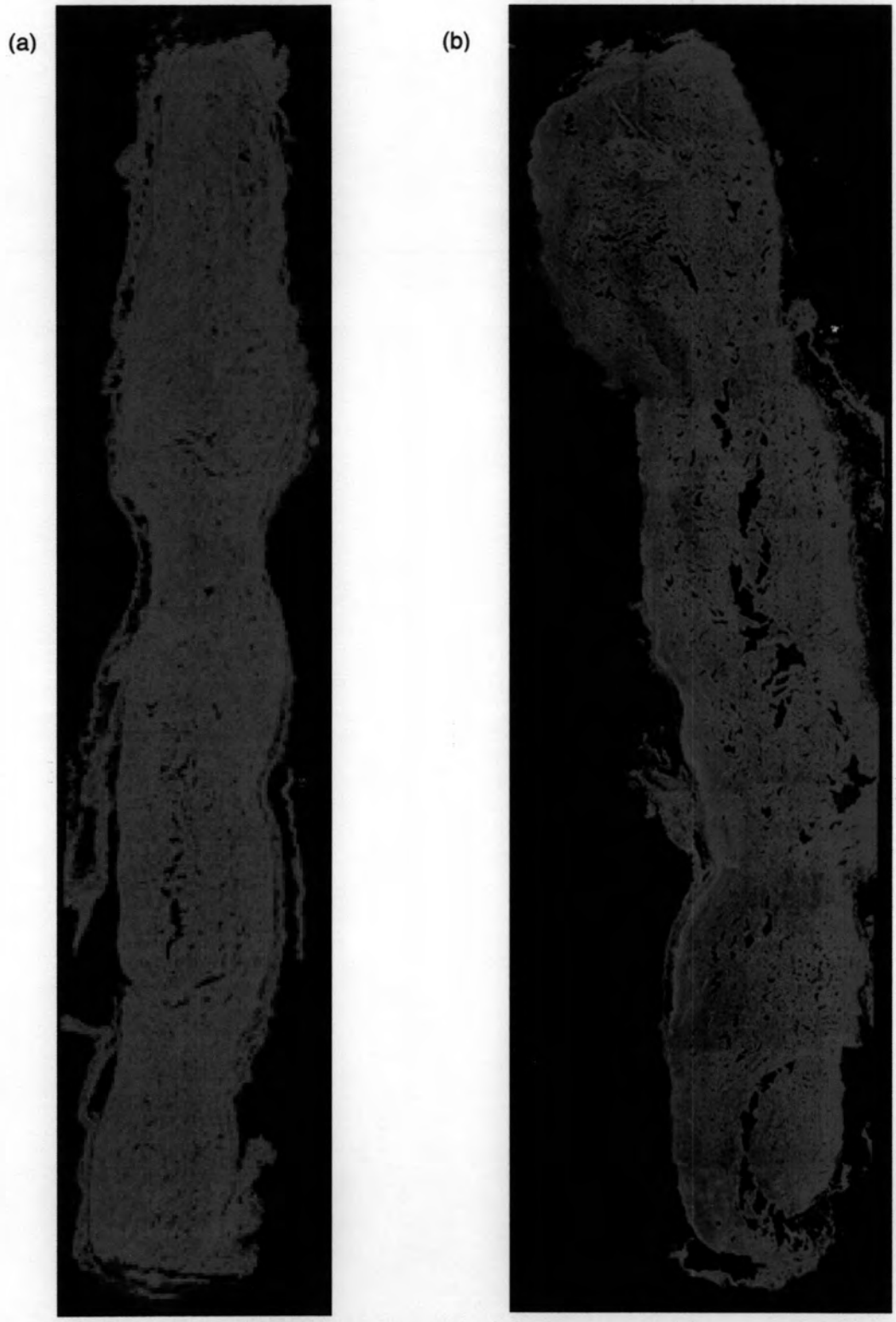


Figure 4.13 Confocal microscopic pictures compared of center section of *in vivo* specimens between hDED (a) and hADM (b) at 2nd week. (100x)

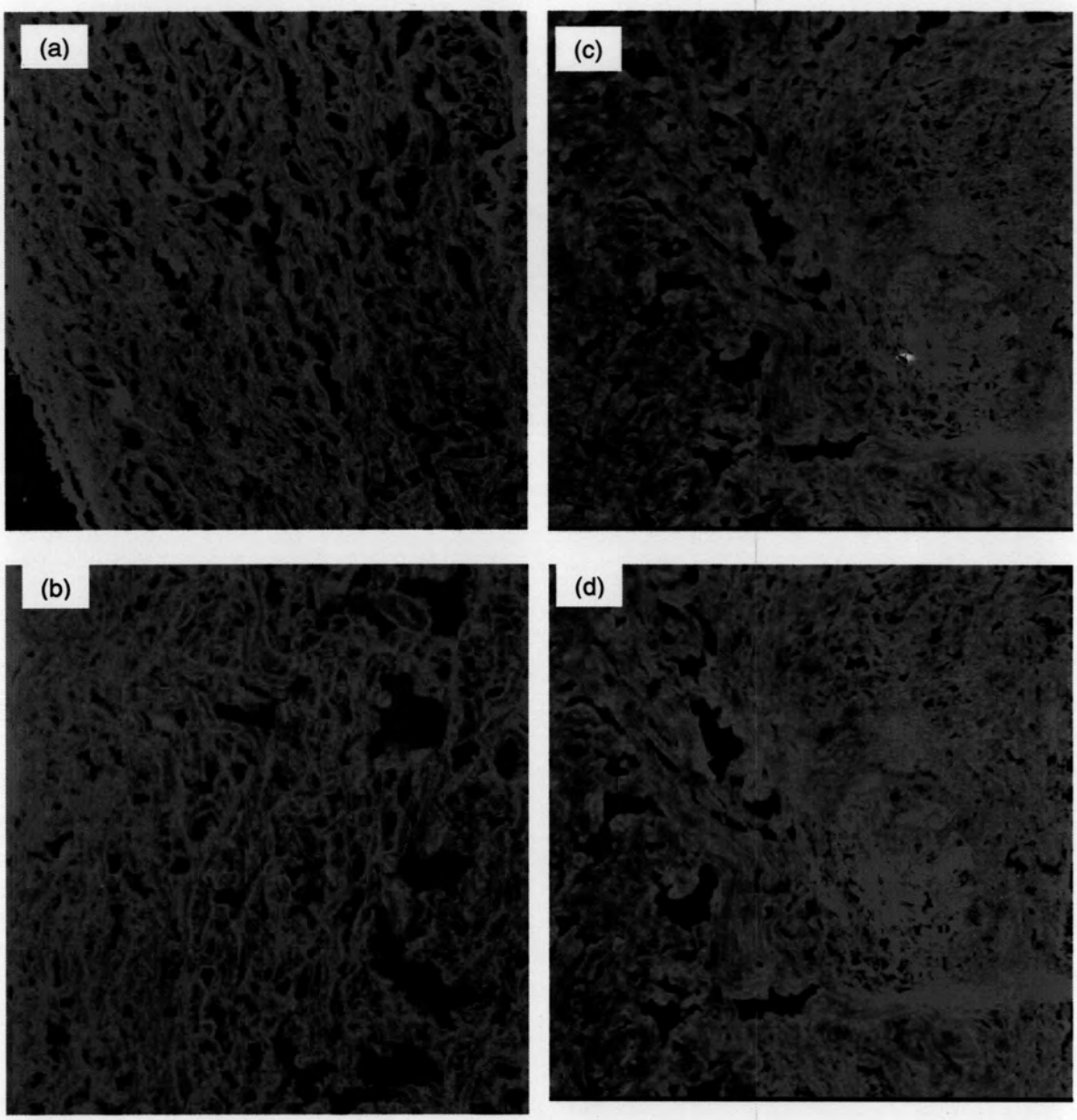


Figure 4.14 Confocal microscopic pictures compared of edge section of *in vivo* specimens between hDED (a-b) and hADM (c-d) at 2nd week. (100x)

4.2.3 The 4th week of *in vivo* study

In the last weeks of investigation, angiogenesis and host-response reaction were gradually decreased compared with the 1st and 2nd week. We could see degradation process of the specimens mostly occurred in hADM (Figure 4.15e) more than hDED group.

In the histological view, in Figure 4.16b, we continuously found dense fibrous tissue; numerous lymphocytes invaded into the center of specimens, and paralleled collagen fibers arrangement. However, we couldn't notice the foreign body reaction in hDED (Figure 4.16a), surprisingly we still found more and more new extracellular matrix deposit from every direction into the center of specimens. As shown in Figure 4.17a, the neo-collagen formation seemed clearer and more complete than in the 2nd week. We could see the perfect structure of neo-collagen in Figure 4.17c and fusiform fibroblasts attached to the old-collagen network to deposit new extracellular matrix in Figure 4.17b. As illustrated in Figure 4.17e-f, detached fibroblast cells, lymphocytes, macrophages, and mast cells were predominated in the specimens. Moreover in Figure 4.17a, dense fibrous tissue and paralleled collagen fibers were clearly seen.

In fluorescence staining, as shown in Figure 4.19a-b compared with Figure 4.19c-d, we could see more fibrous structure of collagen in hDED than hADM. In hADM, collagen structure still appeared in patched and paralleled like the collagen network of scar tissue.

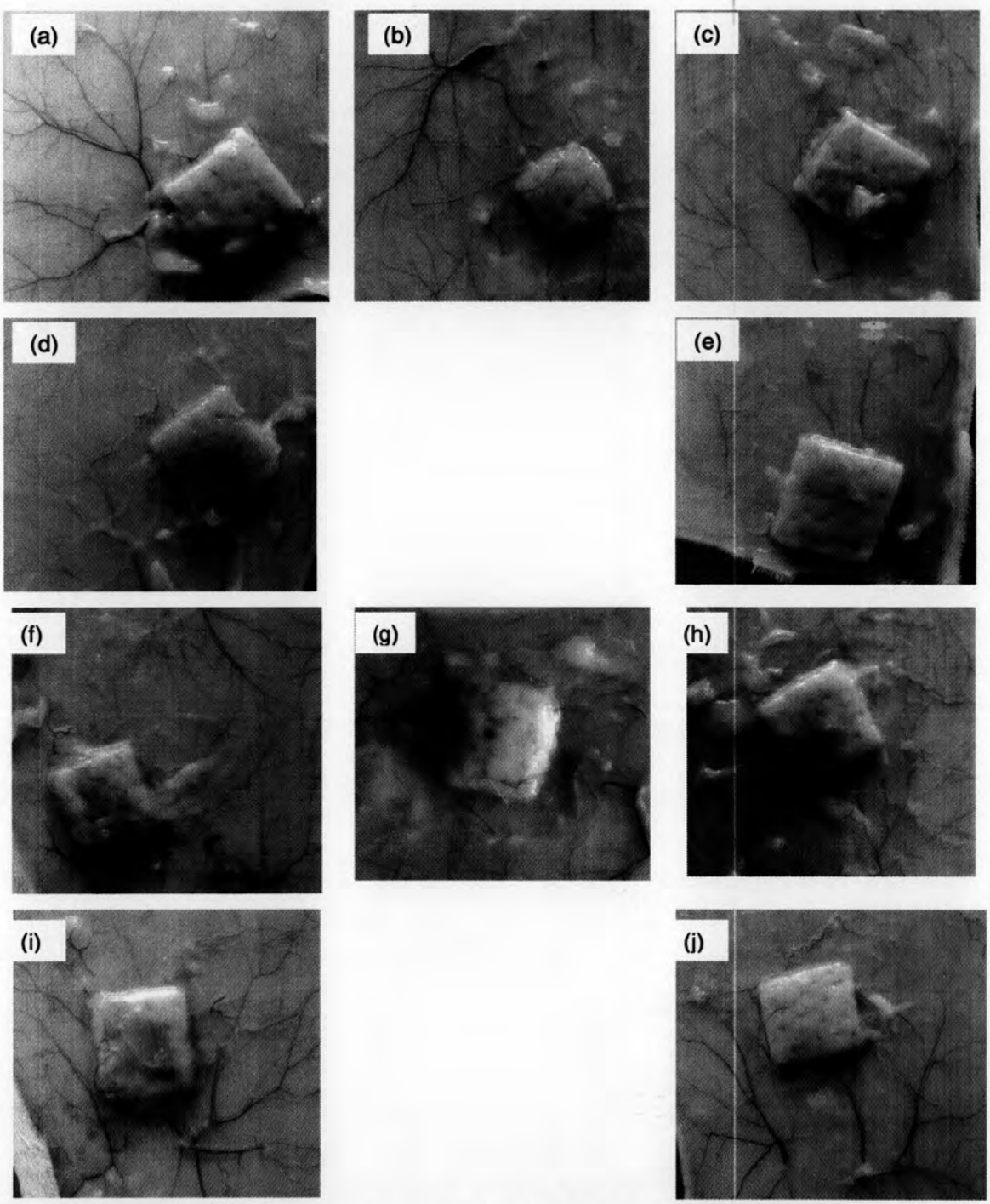


Figure 4.15 Gross specimens (n=5) of hDED (a-e) and hADM (f-j) of *in vivo* specimens at 4th week

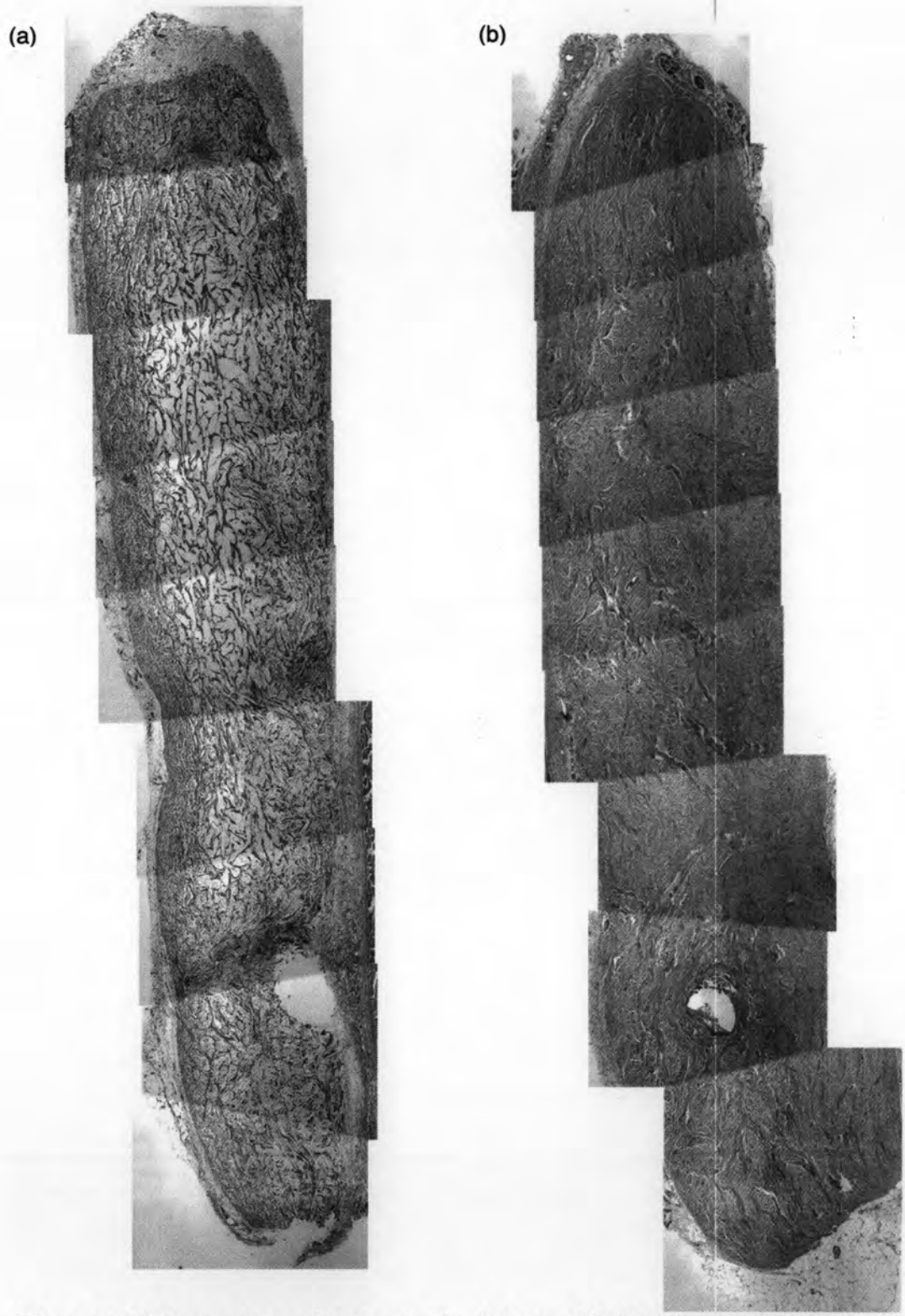


Figure 4.16 Comparison of center section between hDED (a) and hADM (b) of *in vivo* specimens H&E staining section at 4th week. (40x)

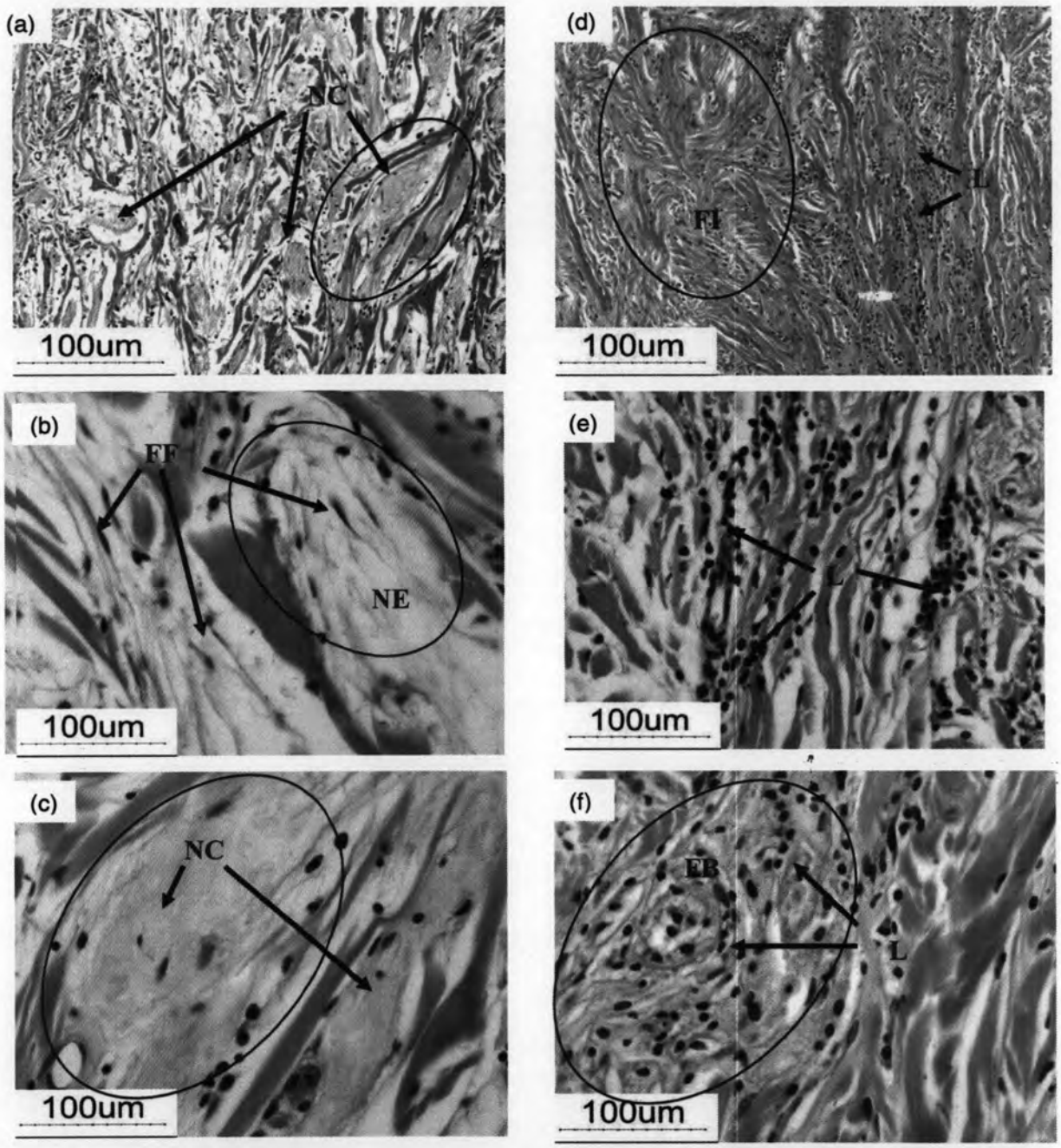


Figure 4.17 H&E staining under light microscope of hADM 100x (a), 400x (b-c) and hDED 100x (d), 400x (e-f) of *in vivo* specimens H&E staining section at 4th week
Fusiform fibroblast (FF), Fibrous tissue (FI), Neo-extracellular matrix (NE), Lymphocyte (L), Neo-collagen (NC), Foreign body reaction (FB)

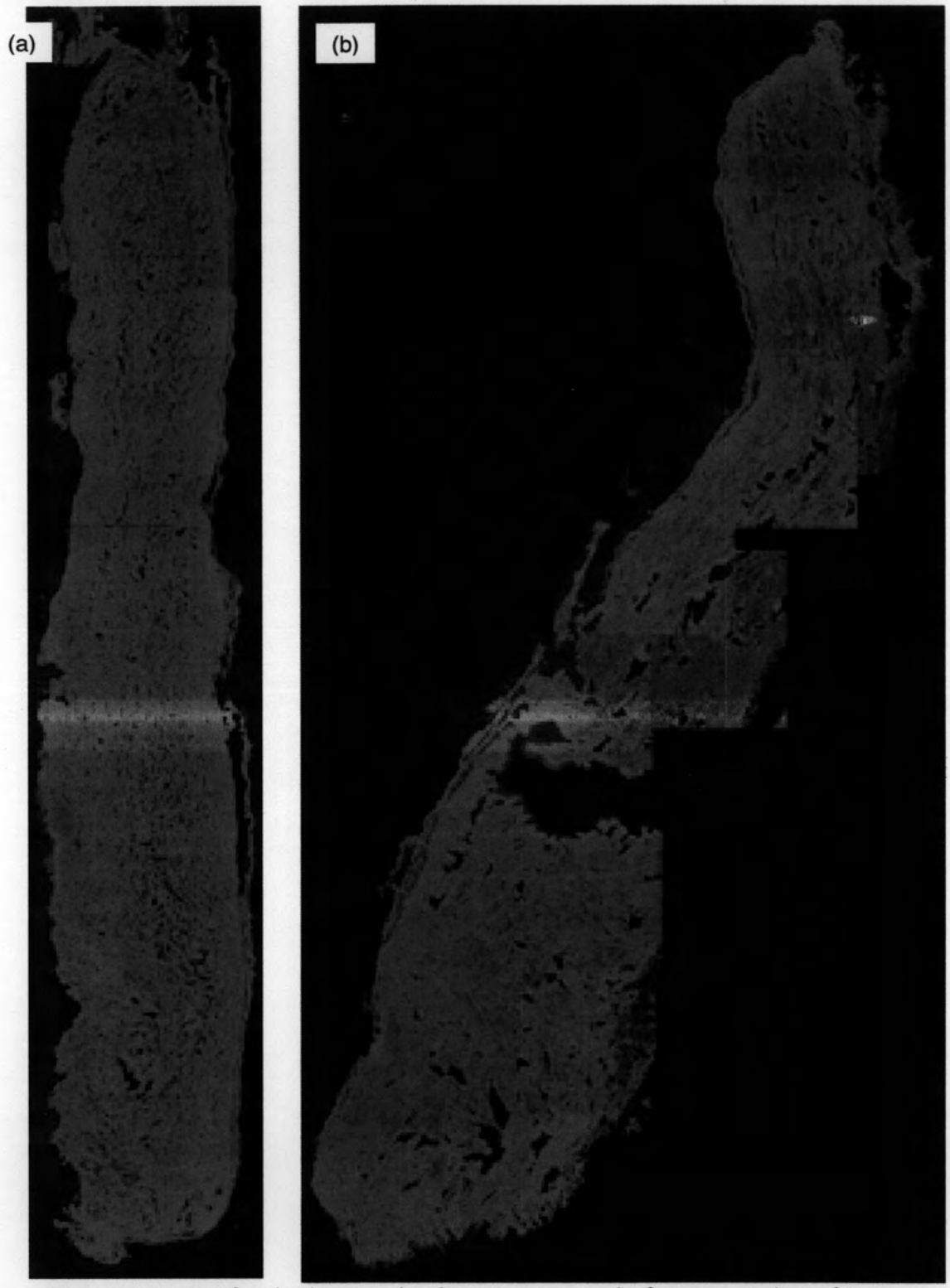


Figure 4.18 Confocal microscopic pictures compared of center section of *in vivo* specimens between hDED (a) and hADM (b) at 4th week. (100x)

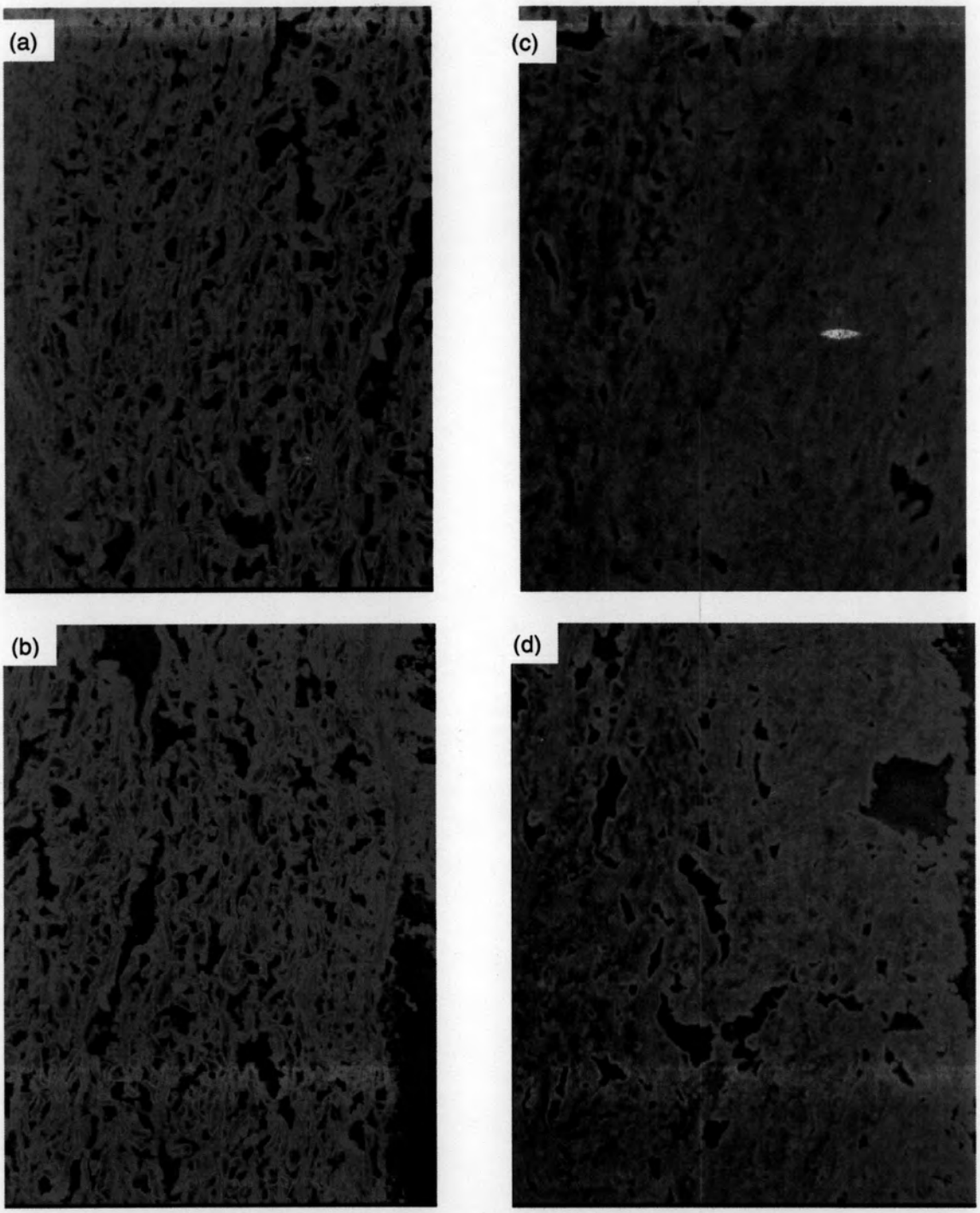


Figure 4.19 Confocal microscopic pictures compared of edge section of *in vivo* specimens between hDED (a-b) and hADM (c-d) at 4th week. (100x)